Investigations in the General Field of

MYCOLOGY.

Continuation of the Moulds and Yeasts,

BY

DR. OSCAR BREFELD.

Part XIII.

Smut Fungi (Hemibasidia IV).

(Continuation of Parts V, XI and XII).

Contents:

O. BREFELD, R. FALCK, Blossom Infection by Smuts and

Natural Distribution of Smut Diseases.

With Two Phototype Plates.

MÜNSTER i. W.

PUBLISHED BY HEINRICH SCHÖNINGH.

(English translation by Frances Dorrance)

Investigations in the General Field of

MYCOLOGY.

Continuation of the Moulds and Yeasts,

BY

DR. OSCAR BREFELD.

Part XIII.

Smut Fungi (Hemibasidia IV).

(Continuation of Parts V, XI and XII).

Contents :

O. BREFELD, R. FALCK, Blossom Infection by Smuts and Natural Distribution of Smut Diseases.

With Two Phototype Plates.

Ordered for this item

MÜNSTER i. W.

PUBLISHED BY HEINRICH SCHÖNINGH.

150 Copies.

Privately Printed and Distributed.

CONTENTS.

Preface.

Introduction.

Blossom infection by smut fungi.

Methods of blossom infection.

Blossom infection in wheat.

Blossom infection in barley.

Infection in oats.

Blossom infection in Melandryum.

Infection of water plants.

The Infection of the Maize plant.

The Infection of Indian millet.

The Infection of Panicum (Rispenhirse) and Italian millet.

Final consideration.

On nitrogen assimilation.

PREFACE.

The present investigations, which have chiefly as subject matter the newly discovered blossom infections by smut fungi and, in connection with them, the natural distribution of smut diseases, form the continuation of the work on smut fungi already published in 3 parts, Vols. V, XI and XII, of this work.

It would not have been possible to carry out these new investigations to the extent here reached, if the Cultusministerium, at my suggestion, had not been so very kind as to grant the requisite means.

It is a great satisfaction to me to be able to express at this place my respectful thanks.

Professor BREFELD.

Breslau, September, 1905.

INTRODUCTION.

The investigations on smut fungi diseases, published in Parts V, XI and XII[1] of this work, date back, in their beginning, to the second half of the 70's in the last century. It was a time when a noticeable standstill had come in the investigations of smuts and smut diseases. More than 25 years had already elapsed since *Tulasne*[2] had carried through methodically the germination of smut spores in water and had ascertained that these spores did not germinate into mycelia, but into short germinating tubes which, becoming fructificative with an early cessation of their growth, form conidia. *Tulasne* designated this fructificative kind of spore germination as germination with promycelia and sporidia. He had observed also an almost corresponding fructificative germination in the talentospores of Uredo and had used here too the same designation by which he had already expressed hypothetically the close connection of both form of fungi.

Meanwhile experimental infection had been carried out with smut spores whose peculiar germination had now been ascertained by *Tulasne,* in order to develop smut diseases in the different proper host plants. These experiments were made chiefly by *J. Kühn*[3] and showed that the mature plants are resistent to and insured against the attacks of smut germination and that infection remains limited to the short stage of the germination of the seed. Disinfection with solutions of copper sulfate, previously carried out with success,—thus killing the smut spores,—obtained here its natural explanation and valuation[4]. The germinating seedlings are attacked in the earth by the germs of infection which come in contact with them. They penetrate quickly through the young tissues into the meristematic tissue of the seedling and continue their growth here, in order to form later the smutted places on the completely matured plants, usually in their ovaries. The place on which the smut appears on the plants attacked lies accordingly as far distant as possible from the place of infection in the young seed. During the period of development of the host plants, nothing may be seen of the effect of the infection. The accommodation of the parasites to their hosts is thus absolutely complete. The parasite appears externally only in the last stage of development.

---

(1) Botanische Untersuchungen über Hefenpilze. Untersuchungen aus dem Gesammtgebiete der Mycologie. V. Heft. Die Brandpilze I. Verlag von ARTHUR FELIX, Leipzig, 1883. Band XI. Die Brandpilze II. Die Brandkrankheiten des Getreides. Commissionsverlag von HEINRICH SCHÖNINGH. Münster, 1895.—XII. Heft. Hemibasidii, Brandpilze III. Commissions-verlag. Münster i. W. 1895.

(2) TULASNE, Mémoire sur les Ustilaginées comparées aux Uréd. Ann. d. Sc. nat. 3 Série Tome 7, 1847—Seconde Mémoire s. l. Urédinées et les Ustilag. Ann. d. sc. nat. 4 Série, Tome 2, 1854. As is well known, PREVOST before TULASNE had observed the germination of smut spores in water and from this had traced smut diseases to fungi living parasitically.

(3) KÜHN, Die Krankheiten der Kulturgewächse. Berlin, 1858, and later works.

(4) The fungicide action of copper on smut spores has also been stated by PREVOST.

The knowledge already won on the one hand by *Tulasne,* concerning the fructificative germination of the smut spores, and *Kuhn's* discoveries on the other hand, concerning the manner of infection of the host plants by the smut germs, were supplemented so simply and naturally that it became easy for the imagination to form a complete picture of the etiology of smut diseases. Smut spores, germinating fructificatively in the earth, press, with the help of the conidia formed, into the germinating seedlings and produce the smut diseases which appear first with the completed development of the host plants.

From the discoveries already existing, scarcely a gap remained for further and new points of attack in order to continue the investigations of smut diseases, and thus it becomes clear, that the advancing understanding of the matter could reach a standstill as has been emphasized already.

My investigations begin here;—I began my observations by investigating the germination of the spores of various smut fungi as far as they were accessible. In those investigations, I had always to convince myself of the striking fact that the *germination of smut* spores is scanty and inactive *in water.* As a rule, only *a part* of the spores germinate and the products of this germination, the conidia of the promycelia, were so passive in their further germination that germination tubes were only rarely formed from them. Besides this, spores of other forms of smut fungi behaved very negatively. They could not be brought to any germination in water; for example, the spores of the prevalent maize smut, which until then no one had seen germinate. In the face of these phenomena of germination, the question was asked involuntarily, how it was possible for these delicate germinating smut spores to reach the host plants and to penetrate into them. This question became still more difficult when extended to the spores which would not germinate at all, such as maize smut spores.

These ever returning phenomena in the germination of spores in water pointed with urgent necessity to the fact that a gap in our knowledge must exist at this place and that without the co-operation of further, as yet unknown, factors for the germination and development of smut spores, the existence of smut fungi and smut diseases could scarcely be thought possible in such general distribution as we see them in nature.

The developmental stages then suspected but still unknown, were found very quickly when I investigated the *germination of the spores in nutrient solutions and substrata, instead of in water.* I had already carried this through successfully for a number of other fungi living parasitically[1]. It became evident first of all that the spores, otherwise germinating singly, were stimulated at once to germination as a whole and that the smut spores which did not germinate at all in water, such as the spores of maize smut, proceeded at once to germination, without an exception. The development in nutrient solutions was as luxuriant as could be observed only in other fungi living saprophytically. Who would have thought, in glancing at the abundant saprophytic development, that developmental members of the most specific of all parasites were involved here, which as yet had been observed only in definite species on living plants, and indeed only in definite parts of those.

(1) Compare the works published in Part IV of this work.

The saprophytic nutrition referred to here took place, not only on one nutrient substratum, but on any which were used for the culture. The parasites accordingly behaved outside of the host plant just as do other fungi which live saprophytically and do not exist simultaneously as parasites.

In cultures in clear nutrient substrata, it was shown further that the conidia formed by the germination of the spores were increased in many places by direct budding without change of form. They illustrate in this manner of reproduction different forms of budding fungi which are characterized by the form of the bud, by the definite place of budding and the early separation of the budded members from one another. These bud conidia, which were increased through unceasing budding until the nutrient substrata were exhausted, are therefore proved here to be released developmental members of our smut fungi, however much they outwardly give the impression of ordinary yeast fungi. Some of these bud conidia are able to continue their budding in the air also and to form conidia there which are disseminated through the air; as, for example, the bud conidia of the maize smut. The germinating spores of the different forms of smut fungi in nutrient solutions are illustrated in Part V of this work[1] and especially the bud conidia, which, belonging there, were then pictured as they are produced at the period of exhaustion of the nutrient substrata.

In Part XII of this work may be found further statements on the germination of smut spores, especially on the *morphologic* decision as to spore germination in promycelia with sporidia. In order to interpret this correctly, extensive investigations on Basidiomycetes, continued for many years, had to take place first;—the results of which are united in Parts VII and VIII of this work[2]. It was shown that the basidia of the Basidiomycetes occur in two different forms; once as definitely organized basidia, each forming one spore from each cell, and then as unicellular, unorganized basidia which produce at the tip a definite number of spores, mostly four[3]. These two forms of basidia, named Protobasidia and Autobasidia, showed a striking correspondence with the two forms of promycelia which were formed in the germination of the smut spores. The formal production of the promycelia in the one type of Ustilagineae corresponds perfectly with the form of the basidium of Protobasidiomycetes and, in the same way, the undivided promycelium in Tilletia with its apically formed sporidia is formed exactly like the basidia of Autobasidiomycetes. The difference between the above named formal types of the smut fungi for the one part, and the two basidia forms of the Basidiomycetes for the other part, consists only in the fact that the number of spores in the former is still indefinite, but in the latter has become definite[4]. The promycelia are therefore nothing but basidia which have not yet reached the higher and typical formation of the basidia. The basidia of the Ustilagineae are accordingly the first stages of the actual basidia in Basidiomycetes. They explain the natural course of morphological differentiation which has held in the formation of the basidia. The organization and

---

(1) Compare the illustrations on the 13 plates in Part V of this work l. c.

(2) Compare with these the illustrations on the plates in Parts VII and VIII of this work.

(3) Compare with these the illustrations on the plates in Parts VII and VIII of this work.

(4) The illustrations on the plates in Parts V and XII should be compared with those in Parts VII and VIII as mentioned above.

formal production in basidia are the same; the difference consists only in the number of spores. Therefore the Hemibasidia of the Ustilagineae have already the characteristic form of the basidia but as yet no definite number of spores. The growth from the first stages up to the complete basidium occurs first in the actual Basidiomycetes. The Ustilagineae are accordingly Hemibasidiomycetes. They pass over naturally in their types, once with divided hemibasidia, again with undivided ones, in one direction to the Protobasidiomycetes, in the other to the Autobasidiomycetes[1].

In the formal sphere of the Ustilagineae with Protohemibasidia, there is the interesting addition in the formation of Hemibasidia that there exist forms, such as those, for example, in *Ustilago longissima* and also in *Ustilago grandis*[2], in which the Hemibasidium has not yet become typical in its formation and in which the conidia always grow out again to irregular Hemibasidia. In *Ustilago bromivora*[3], the hemibasidia have become typical, but the conidia still grow out to hemibasidia. Only in the later forms of the genus, Ustilago; for instance, *Ust. carbo, Ust. maydis, Ust. sorghi (cruenta*[4]*)*, etc., the hemibasidium is limited in the germination of smut spores to the single typical formation with conidia as subsidiary fruit forms, as in typical basidiomycetes. In this limitation the gradtion is shown, through which a hemibasidium attains to its highest formation. Compare the detailed illustrations in Part XII, where the new nomenclature is based on a phylogenetic foundation.

In the entire province of morphology, cases of natural relationship passing from the simple to the more complex cannot be ascertained in as clear and convincing a way as they exist here in the forms of hemibasidiomycetes and actual basidiomycetes. This elucidation of the peculiar formation of the basidia has become one of the now immovable supports of the natural classification of fungi, as based on the comparative morphological foundation in the last VI Parts of this work and completed therein.

This is the unexpected outcome of the germination of smut spores in nutrient solutions from the phylogenetic side, in the consideration of the natural classification of the fungi. It leads to the natural valuation of the basidium and through this alone to an understanding of how the course of morphological differentiation has taken place in the direction of the basidia.

Besides this, however, these new facts have given new and important elucidations of the saprophytic life of these fungi from another point of view. The theory previously held, accord-

---

(1) We are here involuntarily reminded of TULASNE, who designated the fructificative germination in the Ustilagineae and in the Uredineae jointly, on the ground of their formal correspondence, as germination in promycelia and sporidia. TULASNE had observed germination of these spores only in water where no differentiation at all is shown in the promycelia of the Ustilagineae and the Uredineae. The characteristic formal variation existing between these, however, can be determined only by culture of of the spores of the Ustilagineae in saprophytic nutrient substrata instead of in water. Here TULASNE'S promycelia in the Ustilagineae were first proven to be Hemibasidia in contrast to those of the Uredineae, whose promycelia are shown by comparison to be typical basidia, Protobasidia.. As has already been said, TULASNE suspected the natural relationship of both sets of forms, but a determination of the characteristic differences existing between the two types was not granted him. This was first made possible by the improved methods in the culture and investigation of fungi which I introduced and chiefly by the fact that I broke through the conception held until then, according to which the parasitic fungi, and here especially smut fungi, are dependent for development only on their host plants.

(2) Compare plates VIII and IX in Part V.

(3) Compare plate X in Part V.

(4) Compare plates II, III, IV and VII.

ing to which the fungi living parasitically were dependent alone upon the proper host plants for their development, has now been completely upset. Even smut fungi, the most specific of all parasites, have been shown to be only facultatively parasitic. *Therefore all doubt is cleared away and parasitism itself can not be considered to be anything else than a phenomenon which, simply accommodated to circumstances and more or le ss matured, appears in the different forms of parasites.*

The new forms, occurring in saprophytic nutrition, supplement the picture of morphologic forms of these parasitic fungi and make of it an homogeneous whole. Aside from the morphologically biological side first emphasized, the *pathological* especially receives now its true valuation. Only in the new forms, shown in the saprophytic life of our fungi, is it first possible to form a natural idea of how these fungi become effective as parasites. Instead of the few, weak sprouting spores in water, new spore forms appear which mature saprophytically in unceasing abundance and which, because of unweakened energy for development, are able to seize upon nutritive plants and to attack them successfully.

Infection experiments with this newly acquired material of infection germs were planned soon after its discovery. First of all three forms of host plants were chosen for the experiments, —first, Indian millet; second, oats; and third, maize, together with the forms of smut belonging to them[1].

For these infection experiments with the host plants named, at first only budding conidia were used which had been developed in nutrient solutions and kept there more than a month, constantly developing and sprouting. The virulence of this material was tested and, in each case, after budding had ceased, germination of the bud conidia which were to be used produced strong germinating tubes. The bud conidia of *Indian millet* were sprayed on the sprouting seed in dilute nutrient solution by means of an atomizer, in the smallest drops possible. This infection of the young germinating seedlings resulted in the autumn in as much as 70% of smutted plants. For this kind of infection Indian millet is better suited than other experimental plants because of the slow growth of its germinating seedlings and further it was here easily possible to determine the limits within which infection is effective in the growing germinating seedlings. Seedlings when just sprouting were proved to be the most susceptible. Susceptibility decreased with the further lengthening of the germinating seedlings and was ended when they had grown some cm out of the sheath. Penetration of conidia into the germinating seedling, sprayed on as described above, was easily determined by observation, since the penetrating germinating tubes leave a noticeably large hole at the juncture of the two epidermal cells[2]. The further penetration of the germinating tubes through the epidermis into the interior of the tissue may be followed without difficulty.

With the results obtained with this corn and, in the same way with the succeeding experimental plants, it was proved incontestibly that infection germs produced in artificial substrata are most highly capable of infecting the host plants.

---

(1) Compare the discussions in Part XI of this work.

(2) See the drawings on the plates in Part XI.

In the second object under experimentation, *oats,* besides the direct infection of the germinating seedlings by spraying with conidia, a second form of infection was obtained by the introduction of abundant conidia of the oat smut into good compost earth, rich in humus, and also into well manured earth, leaving them undisturbed for some time. The young seed and oat grains were then covered with the soil thus infected and the cultures were left undisturbed in a place not too warm. The sprouting oat grains had to grow through this infected earth layer and, as shown by the experiments, underwent an infection leading to 30 to 40% of smutted plants, harvested later. With these experiments it was proved that the germs of infection living in the soil and particularly in manured earth, where they developed further, can attack the host plants and produce smut diseases as found in nature in fields of oats.

With both experimental plants, Indian millet and oats, the young germinating seedlings are susceptible to infection by smut fungi; in the third experimental plant, *maize,* infected with the common smut, the infection experiments on young seed were without result; only here and there did a young maize plant become smutted or destroyed by a smut swelling. All the other infected plants remained perfectly healthy. They developed into large blossoming maize plants without a trace of disease. Particularly in the pistillate flower spikes of the mature plants, the smut phenomena never occurred. Accordingly, the so universally extensive and striking phenomena of this common boil smut in corn can not be called forth by infection of the germinating seedling of the young seed, as was proved in oats and Indian millet. The parts in which infection takes place are to be found in mature plants and could be found without difficulty in the infection material, the conidia of the corn smut, which was acquired in unlimited abundance and easily used. All parts of the mature plant were proved to be capable of infection, if the young tissue of their new shoots grows sufficiently freely and near the outside. Infection could be obtained by injection of bud conidia into the vegetative tip of the young plant as well as in young leaves, young axillary parts and in the young staminate inflorescences, in forms almost never observed in nature. Quite independent of these places, the embryonic cells of adventitious roots and particularly young pistillate flower spikes, appearing ultimately, are proved to be especially capable of being infected. The adventitious roots were transformed into thick smut swellings. The separate ovaries of the pistillate flower spikes were similarly developed into giant smut boils which under certain circumstances caused the whole infected spike to grow to the size of a child's head[1]. It was shown in further experimental infections that only the very youngest tissues are accessible for infection germs and that the parasite remains strongly localized on the point at which it penetrated into the tissue. After an interval of 14 days, the formation of smut spores occurs at this point in the tissue excrescences already formed. The same places in a somewhat more advanced condition, the tissues being already hardened, are no longer accessible for action of the fungus. It penetrates them indeed but develops neither tissue excrescences nor smut boils. Susceptiblity of maize to the germs of infection is found in all sufficiently young embryonic parts of the tissue, which are accessible from without.

---

(1) Compare the illustrations on plates III-V in Vol. XI.

Accordingly, in the large maize plant, the matter is quite different from that in oats and Indian millet. This plant offers the fungi germs, in the different life stages of its vegetative points, young tissue accessible from without,—for example, in its embryonic leaf buds, young axes, in the adventitious roots supplementarily formed, the pistillate flower spikes etc. In oats and Indian millet these tissues are shut off from the outside and are inaccessible for the germs of infection. For this reason, the young maize plant is not attacked by the germs of infection in the young seed but in the *mature plant*. The parasite which has already penetrated into the tissue remains strongly localized in the place penetrated and every part accessible to infection, in the young leaves, blossoms, axes and roots, must be infected by itself if the smut boil is to be produced which at the latest appears after three weeks.

The etiology of the common smut accordingly differs essentially from that of the two earlier cases and the manner in which infection is carried out in nature is not less different. Smut spores which did not germinate in water, produce bud conidia in saprophytic substrata, in rich, mouldy and well manured earth quite the same as in the translucent nutrient solutions. These bud conidia very soon pass over into air conidia and it is the *air conidia*[1] which easily disseminated through the air reach, without any difficulty, the susceptible parts of the host plant in which we observe the appearance of smut. Further, it is conidia produced by saprophytic nutrition, and especially air conidia, which carry out infection in nature. Entire maize plants grown for experimental infection became smutted without exception if the infection was properly carried out.

The experiments, as far as given in Part XII of this work, ended with these results. Experimental infection was repeated in later years in order to obtain smut material, also, for the purpose of instruction and produced thereby a number of additional results.

It was discovered, in experiments on maize smut, that the susceptibility of the host plant is not limited to one part of the young germinating seedling as had been previously supposed, but re-occurred in the most various parts of the matured plant, and that the young blossoms of the pistillate spikes are particularly susceptible to infection from without. This discovery leads of itself to the closely related consideration, whether this case of blossom infection, as proved for maize smut, might exist only in maize. Evidently it is the young tissues of the pistillate blossoms and of the ovules which in maize can be directly attacked. In unbiased, comparative judgment, nothing stands in the way of the assumption that in other host plants inhabited by smut fungi, the ovaries with pistil and stigma, should also be accessible for infection. They too consist indeed of young tissues which may be attacked freely in the open air by infection germs as are the ovaries of the pistillate flower spikes of maize. If no possible cleistogamy exists in the blossoms they are accessible for the germs of infection from without so far as disseminated by the air. Without doubt, a second place of infection exists here which had entirely escaped observation and had to remain so, as long as the etiology of the common smut in maize was not known. For this reason it was possible for the previous conception, according to which the *young germinating seedlings alone could be infected by smut germs,* and also that mature plants

(1) See figs. 1-9 plate II, Part XI.

were immune to the germs of infection, to obtain an almost dogmatic significance. Without doubt this holds good so far as the vegetative part of the plant is concerned. However, it does not hold good any longer, when the embryonic blossom buds appear and the young ovaries with pistil and sigma now become accessible from without. It would indeed be remarkable if the young ovaries in maize alone should be accessible and the pistillate blossoms in all other plants would not be capable of being infected.

This consideration caused me soon to take up infection of the blossoms in our varieties of grain, which, however, were resultless, since the external conditions were as unfavorable as possible for this infection. Plants infected in the open air, or in the grain fields, were injured by birds or cut down before the seed had been harvested. It was possible to observe only the penetration of the germs of infection into the young ovaries. Beyond this, experiments were frustrated. However, in the infected blossoms, so far as the observations were carried on, no smut appeared that same autumn. My time being taken up by other investigations, I let the experiments stop here, only to resume them later in a different place and with better resources. This was after my removal to Breslau. It became clear from a number of preliminary experiments that, without the assistance of a valued and experienced fellow worker and without the resources of an experimental field, investigation in this direction could not lead to very profitable results.

I found the long wished for fellow worker in my scholar and assistant, *Dr. Richard Falck,* and the Cultusministerium, at my request, generously granted the resources proved necessary for carrying out blossom infection in detail. Thus more than four years ago, we began the new investigation of blossom infection as it exists supposedly in smut fungi, and the following results are the outcome of the work done with my young friend, *Dr. Richard Falck.*

BLOSSOM INFECTION BY SMUT FUNGI.

For our experiments in determining blossom infection in the host plants of smut fungi, those forms come first and chiefly under consideration whose spores are powdery and whose spore masses are easily scattered by the wind and thus distributed. These are, first of all, the different forms of *loose smuts* which occur in our grains,—the loose smut of barley, of wheat and of oats. The characteristically chosen name, *loose smut* (Flugbrand), indicates the distribution of the spores by wind, in nature.

The spores of the loose smut, however, are not the only ones which may be considered for blossom infection. We have seen in maize smut that the spores themselves of the spore masses were not scattered, but that, in the germination of spores on a saprophytic soil substratum, *air conidia* are formed which in the place of smut spores take over the distribution of the germs of infection. And the results of infection experiments with maize smut have furnished the most conclusive proof that these air conidia are at least just as effective for the infection of the host plants.

Aside from maize smut there are still other forms of smut fungi which develop just as diffusible air conidia, in which we must consider the possibility as to whether they too can reach the blossoming plants by means of the air. These are, for example, *Ustilago destruens*[1], and especially the stinking smut of wheat:—forms of Tilletia. In all the cases here named, plants are involved *whose pollen is disseminated by wind* and also at the same time *forms of smut fungi with similar dissemination.*

Smut fungi occur, however, also in the blossoms of *host plants, fertilized by insects,* whose smut spores are not disseminated like the loose smut. An especially striking case of this kind is the anther smut of the plants fertilized by insects. On these host plants, only the anthers are transformed into the spore masses and the spores from these anthers do not have the powdery nature of the loose smut. The smut spores are disseminated very little, if at all, by the air, but are held fast in the anthers, and are first forced out of these by the insects which visit the blossom. The fact can escape no attentive observer that the anther smut in white or light colored blossoms; for instance, *Melandryum album* and *Saponaria officinalis,* is betrayed by a peculiar blemishing of the blossom head by violet smut spores. External proof is here given that insects, visiting the blossoms, force out the anther smut as well as the pollen. They carry over the spores of the smut as well as pollen to the stigmae of the blossoms, where conditions for their germination and further development are to be found in the stigma secretion itself.

We will place the forms of loose smut most prominently because investigation has advanced furthest here and the results arrived at are as forceful as they are convincing.

Formerly only one form of loose smut was differentiated, which was called *Ustilago carbo* and which was thought to live in oats, wheat and barley as host plants. In the middle of

---

(1) See figs. 9-12, plate VI, Part V of this work.

(2) See illustrations on plates XII and XIII, Part V of this work.

the 80's, it was determined by spore culture that the loose smut of oats[1] is an entirely different form from the one existing in wheat and barley[2]. The loose smut of oats, when germinating in nutrient solutions, forms chiefly hemibasidia with conidia, which continue budding indefinitely as long as the nutrient substances of the substrata last. After exhausting the nutrient solutions, the broken down bud conidia grow out into strong, long germinating tubes which were never found in conidia whose spores germinated in water[3]. The loose smut of wheat and also that of barley germinate with hemibasidia, to which the first conidia formed remain attached, growing out into long germinating tubes, but free conidia never appear. An increase of the fungus through budding of the conidia, which takes place indefinitely in the oat smut, is never observed here. Only threads are shown which formed hemibasidia directly. They branch weakly in nutrient solutions and develop only a relative length[3]. The difference between this smut form and that of oats is so convincing that, according to my observations, the loose smut in wheat and in barley must be taken as distinct species. The spores of the loose smut of wheat and barley may not be distinguished from one another. Also no differentiation whatever is shown in the germination of the spores. The question remains open whether the fungus in barley is a still different form from the one in wheat. Anticipating the actual decision, *Rostrup* has designated the loose smut of wheat as *Ustilago tritici,* in contrast to barley smut, to which *Brefeld* had given the name *Ustilago hordei.*

After the natural dusting, that is, the infection of the blossom by smut spores, it is absolutely necessary that the spore masses in oats, in wheat and in barley, should appear *simultaneously with the blooming of the grain.* This takes place in a most striking manner. One can observe indeed that the spore masses of the plants attacked precede somewhat the blossoming of the grain plants, but in any case are present at the time of completest development and are capable of being disseminated, when the blossoms are developed and in full bloom. This peculiar coincidence of the blossoming time of the grain and the ripening of the spore masses of the plants attacked necessarily gives rise to the question, whether any dusting, that is, any infection of the blossoms by smut spores, might have taken place here. And the designation loose smut for this spore mass disseminated by the wind, has already become so significant that one is unconsciously led to consider that there is a natural connection between these smut spores, capable of germination, and an infection of the blossom.

---

(1) Compare the text and illustrations in Parts V (plates II and III) and XII (figs. 25-28, plate VII).

(2) Compare figs. 29-32, plate VII, Part XII.

(3) l. c. text and illustrations of Part V.

METHODS OF BLOSSOM INFECTION.

In order to *carry out the experimental infection,* it was necessary to work out methods approaching most nearly the natural dissemination of the spores. That is, so to imitate the phenomena of nature, that the easily scattered smut spores from the spore masses penetrate with the greatest possible sureness into the blossom of the grain without, however, causing any possible disturbances. For this it was necessary to observe especially that the exact period was reached in which the blossom of the grain to be infected was most widely open, thus furnishing the preliminary conditions most favorable for blowing the smut spores into them. Blossom infection naturally could be carried through only in dry weather, best of all in sunny weather, when the host plants are dry and the spores of the loose smut may be easily disseminated. After various experimentation an atomizer of strong rubber and of suitable size was used for this. The smutted inflorescences were put in it and the opening closed with a connection which ended in a tube with a corresponding opening. Previous tests had shown that by this means the smut spores can be driven from the atomizer in sufficient amount, most finely distributed and comparatively powerfully. The heads or panicles to be infected were then placed in a cylinder, the under opening of which was loosely closed with a wad of cotton, and the spores were forcibly blown into it from the open end. After waiting a little for the spores to settle, the heads were taken again from the cylinder. Supplementary tests of heads thus infected proved that in this kind of infection the smut spores were actually carried into the bloom, so far as the existing condition of the single blossoms permitted. Naturally, the number of blossoms of a head which are open at the same time and make possible this penetration is more or less restricted, according to circumstances. The blossoms on a head do not bloom simultaneously, they are generally most advanced in the middle, while those to be found at the base and near the top open later. From this it is evident that, in a single infection by thus blowing in the spores, only a corresponding part of the blossoms of the head can be effectually infected. Thus, infection exceeding a certain per cent may not be expected here. Several repetitions of the infection of single heads are not advantageous, since disturbances of the normal development of the blossoms may always be unavoidably introduced by the processes.

In nature the conditions for dissemination are incomparably more favorable. Smutted plants, standing in grain fields, do not scatter their smut spores only once when there is enough motion in the air, but constantly throughout the whole time in which the blossoms of the surrounding heads are opening successively. Thus the probability of infection of neighboring healthy plants increases appreciably in comparison with infection in cylinders as already described. Rain belongs among the chief disturbances possibly occurring during infection in nature, that is wet weather which deprives the loose smut of its natural fate and carries its spores down on to the soil, where they are lost for blossom infection. However, too dry and too warm weather can also be unfavorable for infection, since it hastens very much the development and ripening of the grain. Germination of the spores needs also a sufficient amount of moisture.

For the sake of brevity, the designation *"cylinder infection"* will be used in the following for the kind carried out in glass cylinders of tested size by means of the disseminating apparatus already described.

The second kind of infection, which supplements most naturally the one already given, is infection by artificial introduction of the smut spores in the separate *blossoms,* which have just opened or are ready to blossom. This kind of infection by artificial introduction of the smut spores in the separate blossoms necessitates naturally more or less forcible attack upon them. The smut spores are most expediently carried over into the interior of the blossoms by means of a fine brush and here placed upon the stigma and ovary. To carry out this infection most skillful hands are needed, which will not injure the further opening of the blossoms but easily and surely, by means of the brush, will carry the smut spores over on to the stigma and ovaries in the interior of the blossom. We have used with advantage the hands of skillful women. After a little practice they have carried out the manipulation of inoculation with relative delicacy and sureness. Since the infection is here carried out on separate blossoms it is more certain than in cylinder infection and the results are also still more assured by the cutting off and removal of all non-infected blossoms. If this is done with the greatest care and skill, it may be assumed, that each blossom must be infected and that each ovary will be attacked by the germs of infection. However, we find here a number of sources of error which are as natural as they are pertinent. If separate blossoms are passed over rapidly in this infection a rather considerable source of error is given for the later per cent of smutted plants, since only a limited number of blossoms of a head may be infected at the same time. The second source of error is encountered if all non-infected blossoms are not removed. Of course in this form of infection the secondary points may be considered as disturbances which are given already under cylinder infection.

From this it is easy to understand that in infection of separate blossoms the result can be complete only in fortunate cases and that a corresponding loss must be shown in all those experiments where cases of error may have crept in. In any case infection of the separate blossoms is disproportionately surer and more effectual than that of cylinder infection, even if it is inferior to this in the artificial introduction of spores here necessary for infection.

A further circumstance is of especial value for the success of blossom and cylinder infection. This lies in the freshness of the infection material, which in any case must be taken directly from the field and if possible should be taken from the same field for the infection of the plant.

Doubtless the methods of infection here used can be further and better improved. The results given below prove, however, that they are reliable and guarantee a relatively high grade of effectiveness.

Besides infection of the blossoms, *infection of the young seedlings* must be introduced in this same experimental field. It was thought formerly but erroneously that infection would succeed only on young germinating seedlings. In the same way it would now be erroneous to assume that, in experimental plants, blossom infection is the only effective one. Both possible means of infection should be kept in sight. First, infection of the young seedling; second, in the blossom. It is very possible that in the same plants both forms of infection can exist side by side.

It must then be asked which of the two is the more effective in any especial case. Besides this a third case is possible,—that only one of the two infection forms exists alone, either the infection of the germinating seedlings or that of the blossoms. The experimental infection undertaken on young germinating seedlings for supplementation and comparison cannot be made in the same season, in summer, as are blossom infections, and for these the same favorable circumstance does not hold good any longer, that is, the capacity for germination of fresh smut material. It is necessary to make these experiments in spring with smut spores harvested from the field during the previous summer and further with grain taken from the same fields, which are, however, free from smut.

The gathering of spore material, which must remain fresh and uninjured until the next spring, is no easy matter. The spore masses are unavoidably polluted by the air even up to the time of ripening, they are detrimentally influenced by occasional rain and especially by insects which creep into the spore masses, eat the spores and when possible deposit their eggs there. Smut material gathered, without special precaution, from the attacked inflorescences of grain will certainly be damaged in the following spring by worms, often indeed being made useless. Very special precautionary measures and peculiar methods are needed in order to get sufficient amounts of pure smut material in summer with the assurance that it has been protected from all injurious influences. From a long series of experiments, the following method has been proved best for obtaining pure smut material for infection the following spring. Spore material is gathered in sufficient amounts, soon after the breaking open of the spore masses, before any injurious influences have made themselves felt, and is kept eight days in a dry place. Then the smut spores are carefully sifted on to white paper through a fine copper sieve which lets through only the spores. The refuse remaining on the sieve is thrown away. Experience has proved that the smut material thus sieved can be as well kept as possible in this powdery form until the next spring and particularly that no insects will enter the smut spores. These sieved spores are carefully put in a number of small flasks with flat bases, which are filled not more than one-fourth to one-fifth. The wide neck is closed carefully with sterilized paper and the spores are kept in a cool, dry place through the winter. The preservation of spores in many small flasks has the especial advantage that if any injurious influence is present in one small flask, the other spores were protected from it. In fact, in this form, the material to be used for infecting germinating seedlings has shown in every case that it has been well protected so that it can be used in the freshest possible condition. In spring, shortly before using, the spores are put in clean water and thrown about five or six times on a centrifugal sieve. The spores thrown out quickly have been proved to be almost perfectly pure and cultures may be made with these spores in a nutrient solution which show scarcely any pollution throughout the period of culture. The treatment of spores in this form has the additional advantage that, by the day's retention in water necessary for the purification of the spores, they are prepared as favorably as possible for germination, and that spores, which are then sprayed in a dilute nutrient solution on the germinating seedling already prepared, will germinate without loss of time in the drops sprayed on the seedling and can penetrate into it directly. For this infection of germinating seedlings, it is not advantageous to use conidia which appear in spore germination and increase almost indefinitely by budding

in the nutrient solution. It is less tiresome and more certain of results to carry out the infection with smut spores instead of conidia, if they have been prepared beforehand for direct germination in the way stated above. This use of smut spores for infection becomes indeed a necessity if no conidia occur in spore germination when one can depend only on the use of spores, as may occur in smut spores of barley and wheat.

The same spore material used in spraying germinating seedlings may now be used in still *a second case,* in order to infect sufficiently the best compost earth. This earth was mixed in the *third case* with horse manure. The spores were abundantly blown on to it with the atomizer and mixed in and the earth thus infected was used for covering the sown grain. In the *fourth case,* independent of these three cases, the grain in a dry condition was mixed with dry smut spores and then sown.

BLOSSOM INFECTION IN WHEAT.

We will now turn to the experiments made in the last four years with forms of loose smut, and their detailed results. We will begin with *experiments on wheat* and the loose smut belonging to it, which *Rostrup* named *Ustilago tritici*. Wheat is a plant well suited for blossom infection. The blossoms of the different varieties open differently, but the stamens generally grow free and the openings and cracks formed between the glumes are wide enough to make possible infection by spores. When inoculating the separate blossoms, only very little work is necessary in order to introduce the smut spores into them by means of a brush. Care was always taken that a larger number of spores was introduced into the blossoms in order to insure infection by this means. The stigmas do not extend far enough out to make possible a limiting of the infection to them alone. Since, however, there are present in the ovary itself young tissues in themselves accessible for the germs of infection, it is not very important if the possibility of carrying out the infection separately on the stigma and on the ovaries is very much restricted. After infection has taken place, the single heads on which the blossoms have been infected, the non-infected buds having been removed, were marked with colored, non-fading threads in order to distinguish them and to make certain their harvesting when ripe, later in the autumn. A record was kept of the single forms of infection and in it were entered at the same time minor details, weather and air temperature. On the third or fourth day after infection some of the infected blossoms were investigated in order to determine how the introduced smut spores had behaved. It was possible to observe with certainty that almost all spores had germinated in the stigma secretion, especially on the feather-like stigma itself, and that long threads extended from the germinating spores, which had sprouted out on the stigma tissues, and were lost among them. Difficulties arose in later observations, undertaken to follow still further the penetration of the germinating tubes through the stigma, since a clear differentiation of the fine threads of the fungus decreased gradually; thereby the growth of the tubes through the stigma into the young ovary could not be seen with certainty. There is, however, nothing against the assumption that the germs of infection, germinating luxuriantly on the stigma, and growing down into it by means of their tubes, reach at last the ovaries themselves. The same may hold good for those spores which germinate directly on the young ovary and penetrate its young tissue. Nothing more than the above given details can be learned by microscopic observation. The ripening of the young grain was watched with great care, and, when ready, it was harvested just as carefully. The harvested heads were kept in a dry place and hung up in loose bags for later ripening. Judging by external appearances, from all the infected blossoms, only healthy grains were harvested, in no single case of which a trace of smut was found.

Besides the infection of separate blossoms, cylinder infection was now undertaken in wheat. The heads on which this was carried out were especially marked with colored threads in order to distinguish them in harvesting. After cylinder infection, the microscopic examination of the blossoms for introduced smut spores was not omitted, as well as the ascertaining of their germination on the stigma.

In this cylinder infection also the appearance of smut never showed in the grain heads harvested in the autumn. The harvested grains preserved with the precautions already given had a perfectly healthy and normal appearance. These experiments were carried out principally on summer wheat; less often on winter wheat, as is shown in the following statistics.

The grains, harvested from infection of separate blossoms and from cylinder infection and especially taken from different varieties of wheat, were sown in the following spring. This was done with precautions which excluded all chances of error. The grains were sterilized with copper sulphate solution according to *Kühn's* process[1] in order to kill all smut spores which might be present on the outer surface. That this actually took place was proved by special experiments in which smut spores were treated according to the same process and at the same period with the same copper solution. After thorough purification they were tested in nutrient solution as to their capacity for germination. When the outer surface had thus been sterilized, the grains were sown in special germinating cases, a suitable distance apart. Each germinating case held about 300 grains. The single grains lay free on a substratum of sterilized vitreous sand, which covered the underlying substratum, 1-2cm thick. The germinating cases were covered and put in a cool place, the germination of the grain was watched. When the sprouting seed had grown possibly 2cm out of the sheath, the cultures were put in the open air in a protected place and then transplanted singly to the experimental beds in the open ground. It is impossible in this kind of treatment for any infection germs to penetrate from without to the young plants. The plants are immune, if the seedlings have grown 2-3cm out of the sheath, as had been proved earlier for sorghum[2], the first green leaves having appeared. In this condition, assured against all external attack of fungus germs, they are planted in the open ground, where they need protection only from frost and other injurious natural conditions. The plants already set out developed in different years quite normally, just as did the remaining grain plants in the fields. They seemed externally perfectly healthy and did not show a trace of disease. Only at the beginning of the blossoming time were results of the previous infection to be seen in places where the embryonic heads grow out of the tips of the surrounding embryonic leaves. These results are summarized in the following tabulated survey. It is necessary here to emphasize only the fact that in the experiments of a separate infection of the blossoms, the damaging smut increased up to 100 per cent. The appearance offered by these smutted fields was very phenomenal; for the experimentor indeed a very enchanting picture, because it proves the success of his tiresome experiments and the correctness of the train of thought previously carried out. Never indeed have such smutted fields been seen, as shown for example in the photographic picture of wheat, fig. 2, plate I, which proves a total infection of all the experimental plants. If in the separate experiments all the plants did not become smutted, it may be traced back to the sources of error already indicated which are unavoidably present in experimentation. But even such fields, in which 50-70 per cent of smutted plants may be counted, give sure and unassailable proof of the correctness of the decision that here, in the loose smut of wheat, infection

(1) After treating 12 hours with 5 per cent coppersulfat solution at 15-20 degrees C., the seed was washed, let stand five minutes in fresh lime water, washed again and then sown directly on the land.
(2) Compare the text in Part XI.

takes place in the blossom. A summary of the experiments thus carried out is added in small print at the end of this section.

Hence it is proved positively that young ovaries are directly attacked on their stigmas by the germs of infection scattered by the wind; that the smut, however, is not developed in the same year; but that rather the germs of infection which penetrated into the young embryonic fruit remain latent in the ripened grain and after the dormant period of the seed grow out in these, equally, with the germination of the embryo, in order to pass over in the inflorescence to the production of the spore masses.

The seed from cylinder infection was treated in the same way. The percentage of smutted plants fluctuated between 18 and 26 per cent. In the survey at the end of the section, the details are summarized in small print from the list of experiments which we made.

The seed harvested in the separate experimental infections was not used up entirely in the experiments, but a part was always kept over in order to answer any subsequent questions. The seed, obtained in autumn from a complete infection of the experimental plants, was *investigated first of all microscopically,* in order *to prove the presence of fungus germs in the grains.* This was done without any difficulty. Mycelial threads of the fungus were found in different parts of the grain, especially underneath the gluten cells. They were especially present near the scutellum. In the germinating seedling, the fungus threads were more clearly distinguishable, since the grain attacked by them and sterilized had been sown, and fungus threads appeared clearly in the tissue cells in all parts of the sprouting embryo, from the scutellum to the vegetative tip. Accordingly no doubt can exist that the germs of infection, which had penetrated into the young ovary, had remained in a purely vegetative condition here and had passed through the dormant period of the seed. They awoke to new life simultaneously with the sprouting germinating seedling and developed equally with this, as it grew into the mature plant. Here it formed anew its spore masses in the inflorescences. Only the threads of the fungus which reached the vegetative tip of the plant with its embryonic inflorescences become fertile at this point and form spore masses. The mycelial threads in all other parts of the plant remain sterile without fructifying. They are widely separated from one another by the growth of the plant and are found with difficulty in the elongated internodes, but very easily in the cells of the nodes, which are often completely filled with them[1]. It is possible for them to redevelop and to cause disease in the axillary sprouts only in those cases where such sprouts are formed on the nodes, and young tissues are developed[2].

Such are the results of blossom infection by the loose smut of wheat, in contrast to which we must now place the *results of infection on the germinating seedlings* of the young seed. Infection of the young germinating seedlings was tried in four different ways as stated above. *First* the grain was mixed with smut spores and then sown directly upon the land. The infectious action of the smut spores clinging to the grains had to take effect here. *In the second case* the young germinating seedlings were sprayed with spores which had been purified and then prepared for germination in diluted nutrient solution. Here the contact of the germinating tubes, growing

(1) Compare illustration 7 on the first plate in Part XI.

(2) Compare text in Part XI, pages 85-90.

out of the smut spores, with the young germinating seedlings was direct and extensive. *In the third case* the dry seed was covered with infected compost. *In the fourth case,* with compost which had been mixed with one-half its volume of *sterilized fresh* horse manure. In the last two cases the effect of the manured compost must have made itself felt. In order to judge correctly the effect of these forms of infection of the germinating seedlings by means of spores of the loose smut in wheat, we must investigate first of all the germination of the smut spores in water and in nutrient solution. The material kept from the previous year, through the whole winter, weakens somewhat in germinating strength and germinates only very slowly. In spore material which is not preserved with special care no germination whatever occurs[1]. The germination of the smut spores of the loose smut in wheat is not fructificative[2]. No conidia are formed, but only germinating tubes which sprout from the cells of the hemibasidia. No increase of the germs of infection therefore occurs here, not even when nutrient solutions are used. Because of this the strength of the infection of young germinating seedlings by smut spores is more restricted in the soil than in other smut forms which germinate fructificatively. In compost and manured earth this made itself especially felt, since here infection can result only through the germinating tubes of the hemibasidia in direct contact with the grain. The chances left for a successful infection of the germinating seedlings, according to the condition of spore germination of the material gathered the previous summer, are from the very beginning, so far as summer wheat is concerned, strongly decreased and improbable. It is scarcely possible to understand how smut spores from the preceding vegetative year, which had germinated weakly in the spring or not at all, can bring about infection of the germinating seedling.

In fact all the experimental infection tried with summer wheat resulted negatively. *From all the plants inoculated as young germinating seedlings there developed only entirely healthy individuals free from smut. The infection of the young germinating seedlings remained therefore unsuccessful even in most varied and numerous experiments, as may be seen from the following survey printed in small type.* It should not be assumed that more favorable conditions for infection can exist in nature than were created and used in our experiments. We are therefore justified in drawing the conclusion that the infection of the young germinating seedlings is little or only slightly probable in nature. We consider our experiments in this line as not yet completed. We will continue them in the next few years and will consider further any possible contingencies.

The experiments in the forms given were attempted also in autumn with smut material gathered about four months earlier, in which the germinating strength of the spores had decreased but little. It was just as impossible to produce any smutted plants by this infection.

Therefore in the loose smut of wheat we face the fact that blossom infection is fully and indeed totally successful and that infection of the germinating seedlings is resultless. We must conclude from this that, in this loose smut, blossom infection is the ruling form of infection, if not the only one.

---

(1) Compare the results of the investigation on germination already given in Part XII.

(2) Compare the illustrations on plate VII, Part XII.

COMPARATIVE SURVEY OF THE INFECTION EXPERIMENTS CARRIED ON WITH THE LOOSE SMUT OF WHEAT.

A. INFECTION EXPERIMENTS IN 1903.

I. BLOSSOM INFECTION.

1. On a hot day, the 19th of July, 1902, wheat heads in full bloom were infected in a blossoming wheat field in Gräbschen. The smut was taken fresh from the same field and was introduced into separate blossoms by means of a brush. Blossoms insufficiently developed were removed. In the next year there developed from the harvested seed

220 stalks, 67.7 per cent of which were smutted.

The control field of the same wheat taken in 1902 from a field free from smut showed no smut.

2. Blossoming bearded wheat in another field was infected in the same way. From the seed harvested

80 stalks developed which contained 66 per cent smutted ones.

II. CYLINDER INFECTION.

1. June 1, 1902, soon after a thunderstorm, cylinder infection with fresh smut from the same field was made in three different parts of a blooming wheat field in Leerbeutel. From the sterilized seed

in place 1, 520 stalks were produced, of which 39.1 per cent were smutted.
" " 2, 549 " " " " " 37.5 " " " "
" " 3, 216 " " " " " 11.6 " " " "

On an average, 29.4 per cent of the stalks were smutted.

The control field, with about 1,000 plants from non-infected seed of the same field, had only two smutted stalks in 500.

III. INFECTION OF THE SEED.

1. Wheat from Münster was mixed with smut spores from the same field and sown. Out of
300 stalks developed there, none were smutted.

The control from sterilized seed of this same field had 2 smutted specimens.

2. Wheat from Leerbeutel mixed with smut spores from Münster furnished
280 stalks with 2 smutted specimens, as did the control also.

3. Wheat from Leerbeutel mixed with smut spores from the same field gave
250 stalks and 3 smutted examples.

In the control, one smutted stalk was found.

4. Wheat from Schlanstedt mixed with smut spores from the same field gave
310 healthy stalks, as did the control also.

IV. INFECTION OF THE SUBSTRATUM.

1. Sterilized horse manure was infected with pure smut spores from the wheat field in Münster, by spraying with an atomizer, and was then mixed with two parts of compost. Wheat from Schlanstedt was sown in the soil thus treated.

Out of 250 stalks started in germinating cases and later transplanted into the open ground, not one was smutted.

2. Smut spores from Münster were mixed with compost and wheat from Schlandstedt was sown on this substratum.

Out of 250 stalks none were smutted.

3. The same experiments was carried out as in 1 and 2 with wheat from Leerbeutel, and smut spores from the same wheat field. This lot, as well as the control, had 3 per cent of smutted stalks.

B. INFECTION EXPERIMENTS IN 1904.

1. BLOSSOM INFECTION.

1. Single blossoms of suitable heads were infected in a blooming wheat field in Gräbschen with fresh dry smut of the same wheat, in two separate places.

The sterilized grains were planted in March, 1904, on sterilized sand from the river Oder, in closed germinating cases and transplanted in April to open ground.

The grains harvested from place 1 grew

93 stalks, 31 per cent of which were smutted.

Those harvested from place 2,

120 stalks, with 58 per cent smutted.

2. A variety of wheat from Gräbschen which blossoms later was infected in the separate blossoms on July 15, in three different places. Fresh smut was used, however, from the field of Schlanstedt wheat. Young germinating seedlings from the sterilized grain were grown in March, 1904, on sterilized sand from the river Oder, and transplanted on the 21st of April, to open ground.

Seed bed 1 furnished 169 stalks, of which 68 per cent were smutted.
" " 2 " 168 " " " 60 " " " "
" " 3 " 267 " " " 85 " " " "

3. Just as in the first experiments, Schlanstedt wheat was infected in July, 1903, with smut spores of the same kind in the separate blossoms and the sterilized grains were treated as above.

Of 171 stalks thus grown, 61 per cent were smutted.

4. Schlanstedt wheat was infected on July 16, 1903, with fresh smut spores from the field of land wheat (Landweizen) from Gräbschen, the harvested seed being treated as above.

Of 160 stalks, 62 per cent were smutted.

5. On July 23, 1903, blossoming heads of land wheat were infected in Gräbschen with fresh smut spores of the same variety, which were finely divided in very dilute malt. The liquid containing the spores was introduced with a fine brush into the separate blossoms shortly before they opened.

The harvested seed was sterilized, planted in March, 1904, on sterilized sand from the river Oder, and transplanted April 21st to open ground. The infection was carried out in three different beds.

The seed from bed 1 developed 129 stalks, of which 81 per cent were smutted.
" " " " 2 " 204 " " " 96 " " " "

(Fig. 1, plate I, was taken from this lot.)

The seed from bed 3 developed 140 stalks, of which 78 per cent were smutted.

6. Finally, Koström wheat was infected in its blossoms under the same conditions as the varities above named.

a. With smut spores from the same variety.
of 175 successfully developed stalks 51 per cent were smutted.

b. With smut spores from a field of Schlanstedt wheat;
of 53 stalks, 63 per cent were smutted.

c. With fresh smut spores from the same field which were sprayed on in malt;
of 73 stalks, 38 per cent were smutted.

In all these experiments, controls were grown from sterilized seed from the same fields. In all, either no smut developed or an isolated smutted specimen occurred only once in a while.

II. CYLINDER INFECTION.

Wheat from Leerbeutel, just beginning to blossom, was infected in the cylinder, July 4, 1903, with fresh smut spores from a wheat field in Gräbschen. The seed was sterilized and in April, 1904, sown directly in open ground. The infection was carried out in two different places. The seed harvested in 1904 was planted in two separate beds.

In bed 1, of 442 stalks, 19 per cent were smutted.
" " 2, " 625 " 24 " " " "

A control of non-infected heads from the same field gave 0.4 per cent of smutted stalks.

III. INFECTION OF THE YOUNG GERMINATING SEEDLINGS.

1. Young germinating seedlings of a variety of wheat from Leerbeutel were sprayed with smut spores of wheat from Münster, which were just ready to germinate, and distributed in very dilute nutrient solution. Half of the seeds grown in germinating cases were then planted on good, well-manured farm soil, and the other half on not very fertile sandy soil.

In the good soil, 0.3 per cent of 480 stalks were smutted.

In the poor soil, no smutted example was found in 360 stalks.

2. Young germinating seedlings of Schlanstedt wheat were treated exactly as above described with smut spores from Münster and the seedlings transplanted to good and to poor soil. The same experiments were repeated four times in the same way, from the middle of March to the end of April.

In experiments 1 and 2, out of 400 stalks planted in good and in poor soil, none were smutted.
In experiment 3, out of 240 stalks planted in good soil, none were smutted.
In experiment 3, out of 200 stalks planted in poor soil, 1.4 per cent were smutted.
In experiment 4, out of 250 stalks planted in good soil, none were smutted.
In experiment 4, out of 220 stalks planted in poor soil, 2 per cent were smutted.

IV. INFECTION OF THE SEED.

The unsterilized seed was mixed, as in earlier cases, with smut spores so that each grain was covered with a black coating of them. The grains were sown directly on the open ground.

1. From land wheat from Gräbschen, infected with smut spores from the same field,
   280 stalks developed in good soil, of which 1 per cent were smutted.
   160 " " " poor " " " 2 " " " "
2. The same wheat treated as above with smut spores from a wheat field in Münster, gave
   in good soil, 153 stalks, of which 0.7 per cent were smutted.
   " poor " 200 " " " 0.5 " " " "
3. Schlanstedt wheat infected with smut spores from the same field, gave
   in good soil, out of 320 stalks, no smutted ones.
   " poor " " " 350 " 2 " "
4. Koström wheat infected with smut spores of wheat from Gräbschen, as well as from the same variety, gave
   in good soil, out of 200 stalks, no smutted ones.
   " poor " " " 150 " 1 " one.

Controls from the same seed, from poor and from good soil, which had, however, been sterilized, showed no smut in the cases of Schlanstedt and Koström wheat, either in good or poor soil. Wheat from Gräbschen gave

in good soil, of 250 stalks, 2 per cent smutted ones.
" poor " " 100 " (they were all which developed) 4 per cent smutted ones.

V. INFECTION OF THE SUBSTRATUM.

1. Good compost was sown with the spores of the loose smut of wheat from Gräbschen, on a substratum in the germinating cases. The seedlings were transplanted April 14, 1904, to open ground.
   From 200 matured stalks, 2 per cent were smutted.
2. Koström and Schlanstedt wheat were treated in the same way and transplanted to open ground.
   No smutted specimens whatever occurred for 200 stalks of each variety.
3. The compost which had been infected with smut spores was mixed with fresh horse manure, otherwise the experiments were carried out as under 1. They gave exactly the same results as under 1.

C. INFECTION EXPERIMENTS IN 1905.

I. INFECTION OF THE SEPARATE BLOSSOMS.

1. Blooming heads of a beardless winter wheat were infected on June 16, 21 and 22, 1904, in the separate blossoms with fresh smut from the same field. The infection was carried on in three different places. The harvested and sterilized seed was sown in pleasant weather, in the autumn of the same year, (on the 7th of October), directly on open ground. The seed harvested from the four different places was planted in special beds.
   In bed 1, 256 stalks developed, of which 34 per cent were smutted.
   " " 2, 267 " " " " 43 " " " "
   " " 3, 296 " " " " 33 " " " "
   " " 4, 230 " " " " 16 " " " "
   A control of 1,000 stalks of the same wheat had only one smutted specimen.
2. The same wheat was infected with fresh wheat smut; the spores, however, were finely divided in water and were put in the blossoms with a brush. From the seed, otherwise treated as under 1, in the next year
   200 stalks developed, of which 14 per cent were smutted.
3. The blossoms of bearded summer wheat were infected with fresh dry smut from the same field in 1904 and the seedlings grown from sterilized seed on sterile sand were transplanted April 12, 1905, to open ground.
   450 stalks developed, of which 63 per cent were smutted.
   A control of this same wheat had 1 per cent smutted heads.

II. CYLINDER INFECTION.

1. Blossoming summer wheat (land wheat from Gräbschen) was infected in the cylinder with fresh loose smut of wheat. The harvested and sterilized seed was then sown in autumn of the same year, directly in open ground.
   1,750 stalks developed, of which 8 per cent were smutted.
   An equally large control from seed of the same field, but from non-infected heads, showed no smut.

2. Blossoming summer wheat was infected in the cylinder with fresh loose smut of wheat. In the next year, from the sterilized seed,

2,000 stalks were grown, of which 15 per cent were smutted.

In an equally large control of non-infected heads in the same field, 4 smutted plants appeared.

C. INFECTION OF THE GERMINATING SEEDLINGS.

For this infection seed of various ages was used from varieties in which no loose smut had appeared. Most of the loose smut spores from 1904 were still capable of germination. They were brought to the point of germination by soaking in water and then, finely divided in a dilute nutrient solution, were sprayed on the plants. The germination of the seed and the spraying on the smut spores took place at a temperature of 10°. After 3 days the germinating cases with germinating seedlings were kept at a constant temperature of 5° and, after 14 days, having developed far enough, they were transplanted into open ground.

1. Nöe wheat from 1900-1901-1902-1903 and 1904—250 stalks of each—no smut.
2. Ohio wheat from 1900-1901-1902-1903 and 1904—250 stalks of each—no smut.
3. Lupizer wheat from 1900-1901-1902-1903 and 1904—250 stalks of each—no smut.

Some comparative cases not kept in the basement, but in a warm room, at 15°-20°C. gave just the same negative results[1].

4. Young seedlings of winter wheat (land wheat from Gräbschen) were sprayed in the autumn of 1904 with smut spores of the same year, the germinating power of which had not noticeably decreased. They were treated as above.

Out of 200 plants kept over winter and matured in the next year, none were smutted.

IV. INFECTION OF THE SUBSTRATUM.

1. Seed of different ages was used as in III. The grains were sown in the germinating cases on compost and then covered with a mixture of 2 parts good compost and 1 part sterilized horse manure, 6 cm. deep. The cases were kept a week at 6°-8°C. (being kept moderately damp by means of repeated sprinkling) and then for 14 days in a cellar at a temperature of 5°.

The experiment was carried through from each of the five different years, on each of the three varieties named above. In all 15 cases the results were negative.

2. Finally the same substrata were mixed in the way given above in the autumn of 1904 with spores of the same year whose germinating power was still unweakened and in them were sown grains of winter wheat from a field free from smut.

Of the plants kept over winter and developed in the next year none were smutted.

---

(1) The temperature in the small rooms (Göpperstr. 4) was regulated during the night by a lighted jet.

BLOSSOM INFECTION IN BARLEY.

We now turn to the experimental infection with the *loose smut of barley*[1]. The smut in its outward appearance and in the form of its spores cannot be distinguished from that of wheat.

Blossom infection of the barley plant cannot be carried out so easily as that of wheat. In only a few varieties of barley do the blossoms open in such a way that the stamens protrude. In most cases they remain enclosed in the blossoms, that is, inside the glumes, which, however, are wide enough open to make possible a dusting on of the spores. In infecting the separate blossoms of barley the period must be exactly observed in which these are the widest open, because at this time the introduction of the spores can best be carried out. In any case the attack which must be made on the blossoms for this purpose is much greater than in wheat, and it is self-evident that by this means an impediment to infection is caused. For cylinder infection only the splits are to be taken into consideration which are found naturally in the blossoms. Here too artificial infection is, under certain circumstances, less easy than wheat. As above, for the separate infection, only infected blossoms are left standing, all others are removed and the single stalks marked with colored threads. This last was done also in cylinder infections.

The ripe grain was collected from both series of experiments, kept dry during the winter, sterilized in spring and sown on sterilized vitreous sand, just as was stated for wheat. As might have been supposed, the results of the infection of the separate blossoms were, on an average, not so favorable as those of wheat. Nevertheless, we obtained here, as may be seen at the end of this section from the subjoined comparative survey of our experimental infections, a high percentage of smutted plants, even up to total infections. (Fig. 1, plate 1.)

As shown in the survey, cylinder infection also gives somewhat poorer results than was the case in wheat. At the most they did not exceed 20 per cent. In the separate varieties of barley used for this infection, fluctuations were found, as may be seen from the survey. On the whole, however, the result of blossom infection was approximately as favorable as that with the loose smut of wheat and, favorable conditions being taken for granted, it can be assumed here with certainty that blossom infection is always successful if the smut spores succeed in getting into the blossom.

Infection of young germinating seedlings was carried out in the same four ways as with the loose smut of wheat, with much the same negative result as was obtained with wheat. *In all these experiments healthy plants free from smut were formed.* In the comparative survey are summarized the series of experiments and their results. Accordingly it holds good for the loose smut of barley, as was said above for that of wheat, *that the infection in the blossom is the predominant form of infection of the host plants, if not the only one.* Also the anatomic conditions

---

1. The **loose smut** of barley, concerned here, must not be confused with the covered smut which also occurs in barley, but does not become dusty and remains enclosed in the beard. This smut form, as I have proved by cultivation, differs essentially from loose smut. The spores germinate fructificatively and form conidia, which increase unceasingly in nutrient substrata, in the form of yeast. ROSTRUP has designated this form, from its external constitution, as **Ust. Jensenii.**

in the dormant seed, as in the sprouting seedling, did not differ from those already given for wheat.

COMPARATIVE SURVEY OF EXPERIMENTAL INFECTION CARRIED OUT WITH THE LOOSE SMUT OF BARLEY.

A. EXPERIMENTAL INFECTION IN 1903.

I. BLOSSOM INFECTION.

Only a little seed was harvested from the blossom infection of 1902. This small amount failed completely when sown.

II. CYLINDER INFECTION.

1. On June 18, 1902, cylinder infection was undertaken in Gräbschen on a field of barley just beginning to bloom. In the middle of the heads chosen a few blossoms were open. From the harvested seed

1,590 stalks were grown, of which 10.5 per cent were smutted.

A correspondingly large control with seed from the same field from non-infected heads showed 0.1 per cent smutted.

III. INFECTION OF THE YOUNG GERMINATING SEEDLINGS.

1. The young, just sprouting seedlings of barley from Münster were infected by spraying with smut spores from the same field. The smut spores had been brought to germination by soaking in water and were then finely divided in dilute nutrient solution.

Of the 200 stalks transplanted in open ground, 1 per cent was smutted.

In the control no smutted stalks were produced.

2. Two-rowed barley treated as under 1 gave no smutted specimens. The same was true of the control.

IV. INFECTION OF THE SEED.

1. Five different varieties of barley were mixed with smut spores, which had been taken from a field of each separate kind, and were sown directly on the open ground. From each of them 300 stalks were grown. The production of smutted stalks was exactly the same as in the controls produced from sterilized seed.

B. EXPERIMENTAL INFECTIONS IN 1904.

I. BLOSSOM INFECTION.

1. Land barley (Landgerste) from Münster was infected in 1903 in the separate blossoms with fresh barley smut spores from the same field.

a. Before blossoming.

b. Just when the heads were blossoming.

At the end of March, 1904, the sterilized grain was sown on sterilized sand from the river Oder in closed germinating cases. On April 22, 1904, the young seedlings were transplanted to open ground.

a. From the grains infected before blossoming

104 stalks were developed, of which 17 per cent were smutted.

b. From the grain infected during blossoming

350 stalks were developed, of which 60 per cent were smutted.

The control from sterilized seed from non-infected heads of the same field gave no smutted specimens in 300 stalks.

2. On June 30, 1903, heads of a two-rowed Hanna barley just blossoming were infected in the single blossoms with fresh smut spores from the same field. The seed was sown in 1904 as given under 1.

300 stalks were developed, of which 57 per cent were smutted.

A control of sterilized seed from non-infected heads of the same field gave no smutted specimens in 250 stalks.

3. Land barley from Gräbschen was infected on July 1, 1903, in the separate blossoms, with smut spores from the same field.

a. Shortly before blossoming.

b. From heads just blossoming.

The sowing in 1904 resulted in,

a. 85 stalks, of which 30 per cent were smutted.

b. 162 " " " 78 " " " "

4. Land barley from Gräbschen was treated on July 3, 1903, as under 3b, but the fresh smut had been taken from a field of Hanna barley. The seed, harvested from the infected blossoms and sterilized, was sown, giving in

160 stalks, 66 per cent of smutted ones.

5. A variety of barley from Gräbschen was infected in the separate blossoms on especially favorably developed heads with fresh barley smut from the same field. The smut was shaken up in the dilute nutrient solution and put on with a brush. Sowing of the infected grain, carried out as before, resulted in 1904 in

180 stalks, which with one exception were all smutted.
(Figure 1, plate 1.)

The control of 500 stalks from non-infected heads of the same barley had 2 smutted specmens.

6. A two-rowed, small barley, which had never had loose smut and whose blossoms always remained closed, was infected June 30, 1903, with fresh smut spores from a field of Hanna barley just as the stigma in the artificially opened blossoms had fully developed. The grains obtained from the infected blossoms were sown in 1904 as described above. Of

160 stalks, 77 per cent were smutted.

II. CYLINDER INFECTION.

1. Blossoming barley from Münster was infected in the cylinder with fresh smut spores from the same barley field in Gräbschen. The sterilized seed was sown in April, 1904, directly in the open field.

500 stalks were developed, of which 12 per cent were smutted.

A control of 500 stalks had only one smutted specimen.

2. Blossoming barley heads from a field in Gräbschen were infected in the cylinder early in July, 1903, with fresh smut spores from the same field. The sterilized seed was sown on April 6th, directly on open ground

400 stalks were developed, of which 20 per cent were smutted.

A control of 400 stalks from non-infected heads of the same field had 1 per cent of smut.

3. The blossoming heads of Hanna barley were infected in the cylinder in the beginning of June, 1903, with fresh smut spores from a barley field in Gräbschen. From the sterilized seeds were grown in 1904

200 stalks, of which 9 per cent were smutted.

A control of just as many plants had no smut.

III. INFECTION OF THE YOUNG GERMINATING SEEDLINGS.

1. A variety of barley from Münster, one from Gräbschen and Hanna barley were planted in soil in germinating cases and the equally developed young germinating seedlings were sprinkled as given above with smut spores from the same field, still capable of germination. Of each variety

600 stalks were developed, of which 1 or 2 specimens of each were smutted.

IV. INFECTION OF THE SEED.

Besides the barley from Münster, Gräbschen and the Hanna barley, a fourth variety was mixed with smut spores and sown partly on well-manured farm land, partly on unfertile sandy soil. In each of the 16 experiments

200 stalks developed, among which there occurred only occasional smutted specimens.

Entirely similar results were obtained in a corresponding control in which sterilized seed had been used.

V. INFECTION OF THE SUBSTRATUM.

Four different varieties of barley were grown in germinating cases, partly on good compost, partly on a mixture of compost and fresh horse manure. The compost as well as the mixture with horse manure was infected abundantly with spores of the loose smut of barley. The seedlings germinating in this substratum and developed further were transplanted later to open ground and of each

200 stalks were grown. They were all free from smut.

C. EXPERIMENTAL INFECTION IN 1905.

I. BLOSSOM INFECTION.

1. Blossoming land barley in Gräbschen was infected in June, 1904, in the separate blossoms with fresh dry, loose smut of barley, simultaneously on two different fields, by three women. The sterilized seed was grown in 1905 in closed cases on sterilized sand, then planted out in two separate beds.

In bed 1, 600 stalks developed, of which 58 per cent were smutted.
" " 2, 560 " " " " 44 " " " "

Equally large controls of the same barley had no smut.

2. Blossoming barley from another field was infected on June 28, 1904, with fresh barley smut, in two different places as under 1. The smut spores were shaken in water and put into very dilute nutrient solution. The harvested seed gave in the following year

In bed 1, of 250 stalks, only 13 per cent smutted ones.
" " 2, " 200 " " 16 " " " "

The control had no smut.

3. The blossoms of a two-rowed cleistogamous barley which had never had smut were infected in June, 1904, with fresh smut of a barley (land barley from Gräbschen). The following year

250 stalks developed, of which 30 per cent were smutted.

II. CYLINDER INFECTION.

1. In a barley field in Gräbschen, shortly before blossoming, the still closed blossoms were infected in two separate places on June 22, 1904, in the cylinder with fresh loose smut of barley. The sterilized seed was sown in two different beds.

In bed 1, 540 stalks were developed, of which 9.5 per cent were smutted.
" " 2, 670 " " " " " 9.7 " " " "

2. The same barley was later infected in the cylinder, shortly after it had bloomed.

500 stalks developed, of which 12 per cent were smutted.

A control of the same barley had no smut in 2,000 stalks.

III. INFECTION OF THE GERMINATING SEEDLINGS.

Seed of different ages was used on fields in which no loose smut or very little had been observed. Most of the smut spores used in 1904 were still capable of germination. The experiments were carried out exactly as was described for the experiments of the germinating seedlings in wheat in 1905. There were used

Probsteiner barley from 1900-1901-1902-1903 and 1904,
and Chevalier barley from 1900-1901-1902-1903 and 1904.

In all ten experiments no smut appeared.

IV. INFECTION OF THE SUBSTRATUM.

The experiments were carried out with the varieties named under III, exactly as was described for wheat in 1905, in the same place. Here, too, no smut appeared in all ten experiments.

From the experiments with loose smut in wheat and barley, which thus correspond exactly, the infection of the blossom has become a scientific fact. The smutted appearance due to the loose smut in the grain fields of wheat and barley has brought forward a newly discovered form of infection of which no one had thought before. The infection of the young germinating seedlings which had previously been considered as the only active one, can count for little, perhaps for nothing at all, in comparison with infection of the blossoms. The new fact is of itself of *high scientific value.* Its characteristic nature is first shown by the fact that the infection in its greatest action is entirely withdrawn from observation. *The germs of infection which penetrate to the young ovaries remain hidden in these and even until complete ripening do not develop fructificatively, nor indeed even at the very point where the smut masses are otherwise always and solely formed. Not one trace may be seen outwardly on the infected and harvested seed of any smut infection which has already taken place. The anatomic condition proves that smut spores are present in the seed and remain quiet during its dormant period. The infection is temporarily interrupted by this dormant seed period and, after this is passed, is continued with the further development of the plant. Only in the germination of the seed can the fungus develop*

further, in order later, at the time of blossoming, to cause, as if by a stroke of magic, the appearance of spore masses in the plant which, until then, had seemed healthy. The adaptation of a parasite to its host plant appears here with a completeness not known in any other case in the plant kingdom. Infection extends back to the first embryonic parts of the young plant and the appearance of disease becomes externally noticeable only in the second vegetative period in the last stages of development.

One would naturally think this inheritance, if the infection of the blossoms were not proved with certainty and traced back to the time of the infection and fruiting of the embryonic parts of the young embryo in the ovary of the blossoms. *These facts, as noteworthy as they are important for the biology of smut fungi from a purely scientific side, adjoin the not less important results which are furnished by blossom infection for the practice of the agriculturist, that is, for the struggle against smut fungi.*

It has been definitely proved by blossom infection that smutted individuals in the blossoming grain field form the centre of infection for the plants and of the further distribution of the smut. The spores are disseminated as dust from the spore masses of the plants attacked and are driven by the wind directly on to the blo ssoms of the surrounding plants. They thus get directly into the blossoms and on the stigma, where they infect the young ovary, susceptible to infection. Only in the following year, however, do the phenomena of smut develop from the seed thus infected as we find them in the fields.

Formerly smutted plants had been observed attentively only from one point of view. It was thought then that the smut spores were driven on to the surface of the surrounding grain and on the soil, and that infection in the soil came to full maturity in the germs of the seed only subsequently and only in them. The possibility of blossom infection, in which smutted plants represent a direct and immediate centre of infection for the surrounding healthy plants, had not been considered at all, nor especially that the seed already harvested could have been attacked by germs of infection from the preceding blossoming period.

In connection with the assumption of an exclusive infection of young germinating seedlings it was the firm belief that the struggle against smut could be successful only if the seed with the spores clinging to its outer surface was *disinfected with sterilizing material,* thus killing the spores on this surface. Of what value is disinfection and sterilization of the seed grains now if the grain is infected in the blossom? Evidently none at all. It is not necessary to kill the spores clinging to the outside by sterilization; for, as we have proved, they do not penetrate into the seed. The germs of infection existing in the seed, which had penetrated at the time of blossoming, can not, however, be killed by sterilization. This disinfection is only an external one. *Sterilization is therefore useless for the forms of loose smut in wheat and in barley.* It has already been shown that sterilized grain infected in the blossom has produced entire fields of smutted plants.

The fact here made clear that smutted plants grow from sterilized seed of wheat and of barley is in itself not new. It has been known to the practical agriculturist for a long time from experience, but its correct explanation had not been found. Further, investigations were directed into a wrong channel, since it was always assumed that the *form* of sterilization was

insufficient and incomplete if, as experience showed, smutted plants could grow from sterilized grain. The different statements concerning new methods of sterilization and their effectiveness are the natural results called forth by this erroneous course of thought. If the study of the phenomenon had not thus been carried on from one point of view alone, the question would have been considered from the opposite one; that is, whether we had learned enough of the way in which infection through smut fungi takes place, or whether the assumption, that infection is limited only to germinating seedlings, is far reaching enough.

Until most recently, investigations of smut infection were carried on with the assumption that infection takes place only in young germinating seedlings. The percentage obtained in different experiments on smutted plants may be traced back to the previous blossom infection. The germs of infection were already present in the seed which was thought to have been infected by the sprinkling on of spores, as, for example, in the experiments made by *Otto Rose* in Rostock, on which he reported in his inaugural address, Rostock, 1903, "Der Flugbrand der Sommergetreidesaaten und Massnahmen zur Bekämpfung dieses Pilzes in der landwirthschaftlichen Praxis" (Loose smut of the seed of summer grains and regulations for fighting this fungus in agriculture). We repeated the experiments with different varieties which in *Rose's* experiments had given the highest and lowest percentages of smutted heads. The seed material obtained from the same source was partly sprinkled with spores, partly disinfected, and sown the end of March in the open field. As is evident from the following table of experiments made with different varieties of barley, the results were the same in both cases. The average temperature in the first three weeks after sowing was somewhat lower than in *Rose's* early sowing. Our later work will throw light on the question of the conditions causing the different results in *Rose's* early sowing, and in his late one.

Barley varieties.	Percentage of smutted stalks in Sterilized Seed	Seed mixed with Smut
1. Bestehorn's	0.5	0.5
2. Bestehorn's Kaiser	0	0
3. Chevalier	3	3
6. Golden Drop	0	0
5. Greek, six rowed	0	0
7. Hanna	0	0.5
8. Imperial, loose	0	0
9. Imperial, irregularly arranged	2	0
12. Mandschurei	0	0
11. Moravian	1	1
13. Naked, small blue	0	0
14. Naked, large, 2 rowed	0	0
16. Naked, 3 forked Neapolitan	0	0
17. Oderbrucher	0.5	0.5
19. Probsteiner	0	0
18. Phoenix v. Thillau	0	0

20.	Rice or Fan (Fächer)	0	0
21.	Black Barley	0	0
4.	Erfurter, white	0	0
10.	Kallina	0.5	0.5
15.	Naked, 3 forked, 3 rowed	2	2.5

The discovery that loose smut does not disappear from the fields of grain in spite of sterilization indicates convincingly that, besides the infection of the young germinating seedlings, still another form of infection must exist for our smut fungi, from which an explanation could be deduced for the fact that smutted plants grow from sterilized seed grains. This fact has only now been made clear and in such a natural and simple way that every agriculturist must be interested in learning that the smutted individuals of a field are the direct centres of infection. The germs of disease are carried directly at the time of blossoming from these diseased plants to the healthy ones.

But this explanation would be only one-sided and not very satisfactory for the agriculturist, if it were not possible to set up a different method for fighting the appearance of smut, instead of considering sterilization of the grain in fallible which had been universally assumed to be effective. And what can be the nature of this new protective means of preventing smut? Clearly it is no other than that the field be prepared only with healthy seed taken from fields free from smut and that thus the struggle against smut be undertaken not positively but negatively. It is scarcely practicable to pull up smutted plants from the field in order to prevent blossom infection. Seed must be chosen from fields free from smut and thus prevent the sowing of seed grains already attacked by the smut infection. *Pure seed from fields free from smut must from now on be the means of ending the agriculturist's struggle against smut.* If this is universally and carefully carried through the appearance of smut must of necessity decrease gradually and cease entirely so far as forms are involved which are infected in the blossom.

With this, our investigations of blossom infection in the loose smut of wheat and of barley are concluded for the present. A subordinate question must not remain unanswered here,—which involuntarily becomes obvious,—*the question namely, whether the smut germs of infection found in seed infected in the blossom can remain capable of development for several years.* The solving of this problem strengthens at the same time the certainty of the fact that infection takes place in the blossoms and that the germs of infection exist latently in the ripened seed.

A part of the wheat and barley seed which had been harvested was retained and used for experiments in succeeding years. New sowings were made, once of wheat and once of barley, from the seed grain from which in the first year, after successful sterilization, the highest percentage of smutted plants were produced, but which had lain dormant two years. The seed was sown with all possible care as given above and, from the grain which proved itself still capable of germination, strong plants were grown which, at the time of blossoming in the second year, gave the same picture of smutted experimental fields as described in the first years and reproduced in figs. 1 and 2. All the plants in the separate experiments were smutted. *By means of these last experiments it was proved to be a fact that the germs of infection latent in*

seed remain capable of development through the period of two years. This result justifies the assumption that the capacity for development can last even as long as, perhaps, much longer, than the germinating capacity of the seed itself. To continue the experiments further in this direction would have only scientific and no practical value, since seed more than two years old is never used in practice.

A second subordinate question must not remain unanswered here which is furnished by the external, almost complete, correspondence between the loose smut of wheat and of barley. The question, namely, do two different forms exist here or is the same smut concerned in the attacks on the Hordeaceae, that is Hordeum and Triticum? The detailed cross-infection of barley smut on wheat blossoms and wheat smut on barley blossoms has not yet given sufficiently convincing results. It is, however, not impossible that the very dry summer of the preceding year influenced the infection of the blossoms. Experiments have been begun again from the beginning and it should be possible later to report results concerning this.

THE INFECTION OF OATS.

A third form of loose smut must now be considered in connection with those of wheat and barley, that is, of the Hordeaceae. *This is the one appearing on oats; that is, on the Avenaceae.* Externally this smut resembles the earlier forms in its spore masses and the form of the spores. When cultivated in nutrient solution, however, these smut spores soon show great differences. The loose smut of oats does not *germinate sterilely, but fructificatively*[1]. Conidia of a definite form are *formed from the hemibasidia* by direct budding; these form a highly characteristic species of bud conidia, the broken-down members of which grow out at once to strong, long germinating tubes[2], when the nutrient solution has been exhausted. These tubes will penetrate into the host plants. To the difference in germination is added now a second difference, that of *the period of germination of the spores.* This does not end, as in the two other forms, with the lapse of a year. It continues for several years and the spores, investigated then as to their germinating strength, germinate just as strongly as those freshly gathered.

It is evident that in this smut form we are concerned with spores which, by the energy of their development and the unceasing increase of their conidia in saprophytic substrata, show a power of infection not possessed by the loose smut of wheat or of barley. The latter depend for their infection on young blossoms; that is, stigma and ovule, and have been proved ineffective for the inoculation of young germinating seedlings in the soil. The behaviour of the oat smut takes the opposite course of action; namely, the inoculation of young germinating seedlings, which is considerably favored by the increase of the germs of infection in the earth.

Inoculations made earlier with loose smut of oats and reported in detail in Part XI of this work[3], have shown that undoubtedly the infection of young germinating seedlings takes place here. The effect of the germs of infection sprayed on the young germinating seedling with an atomizer showed that no total infection was obtained by these circumstances,—needing closer investigation,—but only a result of from 7-20% of smutted plants. The experiments, however, with infected compost and with humus soil, mixed with one-half its amount of horse manure and used for covering the seed grain, gave a percentage of smutted plants which had been increased up to 30-40%.

The results obtained previously were repeated in numerous experiments made in successive years, and particularly in the last five. They furnished no better results from inoculation with the atomizer, but in experiments with humus soil and manured garden earth, they increased to more than 60%. A number of subordinate circumstances are present here which favor infection, among them especially the longer development of the germs of infection in the humus soil and manured garden earth here used for covering the seed. Probably a delayed development in the germination of the seed is here beneficial to the germs of infection. But investi-

(1) See plate II, Part V of this work.
(2) See plate III, Part V of this work.
(3) l. c. page 23.

gations, fluctuating as yet in their results, have not been worked out sufficiently. They will be reported in a statistical survey in the next work on smut fungi.

To determine the influence of temperature, experiments were made on the germinating capacity of smut spores and seed grains at constant low temperatures. It was proved that smut spores can germinate in nutrient solution almost at the point of freezing. Germination is thus only relatively delayed, otherwise it is just like that at higher temperatures. However, the seed germinated also at low temperatures, almost down to zero, but very slowly. If spore germination and germination of the seed is proportionately delayed here by lowering of the temperature, it is not possible to understand how effectiveness can be obtained by cooling. Yet some effect will appear if germination is increasingly delayed in older seed, but not to the same extent in the smut spores. It is not advisable to use too low a temperature for experimental inoculation, since development will only be prolonged; on the contrary, it is questionable whether differences of temperature in infected earth and in sprouting grain can favor infection. Further experiments on this have just been made; they were very much impeded, however, in their exact carrying out.

The first experimental infections with the loose smut of oats on the blossoming grain could be made only two years ago. Inoculation of the separate blossoms is possible in this case only with very appreciable interference with and destruction of the blossoms. The time in which the oat blossoms open can be determined only with difficulty and an artificial opening of the blossoms necessitates a separation of the glumes, which are tightly closed. A high percentage of the harvested oat grain from a blossom inoculation was barren and a considerable number failed in germination, probably as a result of the disturbance due to the mechanical injury made during inoculation. The grains ultimately germinating were shown to have weakened in germinating power and most of them withered subsequently. It must be added here that wire worms appeared in the beds and destroyed all that were left, excepting a few plants from which healthy plants were finally developed.

Cylinder inoculation of the oat blossoms with the atomizer was then carried out. Most of the blossoms of the oat panicles which hang downward were infected from below with smut spores by this cylinder method of dissemination. The harvested seed, however, exclusive of some isolated smutted plants, has resulted as yet only negatively.

From the results obtained in inoculating the blossoms of oats with the loose smut, we can indeed draw no final conclusion as yet, but can say this much:—that blossom infection must be only of lesser significance here; and that, on the other hand, infection of the germinating seedlings in the soil, according to results already reported, is so much the more successful. There are, however, a number of experiments where, according to our experience, the occurrence of smutted plants scarcely makes possible any other explanation than that blossom infection must also take place here. The circumstance that the blossoms of the oat panicle do not stand upward, but hang downward, is not so favorable for direct inoculation of the blossoms in nature by smut spores. The disseminated spores are not driven from below upward, but from above downward.

It is worth notice that the results of infection, which we obtained, are thus connected naturally and harmoniously with the appearances displayed in the germination of spores, especially on saprophytic substrata. The increase of the germs of infection in soil, especially in manured earth, indicates a predominant infection of the germinating seedlings. The noteworthy fact also that the spores of the loose smut of oats have retained their power of germination for many years and that they can remain capable of infection for a long time in the earth indicates also an infection in the soil.

The different results obtained here, on the one hand with loose smut of the Hordeaceae, on the other hand with that of the Avenaceae, should prevent the laying of too strong emphasis on any single factor of infection. Externally the loose smut of oats does not differ essentially from the other two forms. In its biological behavior is first shown the dissimilarity which would have escaped observation when judging only by the character of the loose smut, as was done earlier.

BLOSSOM INFECTION IN MELANDRYUM.

The smut forms already described appeared in the blossoms of grasses; that is, of plants characterized by *wind fertilization*. There are, however, a number of smut fungi which occur in plants fertilized by insects and which attack separate parts of their blossoms. An especially characteristic form of this kind is given in the anther smut, which appears chiefly in the blossoms of the Caryophyllaceae. The infected host plants appear externally absolutely normal, the anthers alone are attacked by the smut fungus, *Ustilago antherarum* or *Ustilago violacea*[1].

Instead of pollen grains, as in normal anthers, thick spore masses with violet spores are found in the pollen sacs here. The spore masses are very abundantly formed and pushed forward from the place of formation in such quantities that the anthers rupture, exposing the spore masses. The spores are not as dusty nor as easily disseminated as those of the loose smut. They have a rather sticky nature, such as belongs to the pollen of plants fertilized by insects. If, for example, the blossoms of *Melandryum album* infected with anther smut are observed for several days at the beginning of their time of flowering, it will be seen that influences are felt here which force the spores out of the anthers. The white inflorescences look as if soiled by clinging smut spores. It is by this means that anther smut usually makes itself known outside of the attacked blossom. The blossoms of *Melandryum album* open in the evening and remain open in the dark. They are visited by insects, especially night-flying butterflies, which stick their probosces into the blossoms, in order to suck the honey. In this way spores of the smut are forced out simultaneously, thus soiling the white inflorescences with the dark smut spores. When convinced of this fact, one understands involuntarily that the forcing out of the smut spores from the anthers of the infected blossoms is brought about by the *butterflies. The infection, that is, the distribution of the smut disease, therefore is not brought about here by the wind, but by insects which fertilize the blossoms.* The insects which have visited a smutted blossom carry over to the stigma, the style and the young ovule of neighboring pistillate blossoms, the smutted spores sticking in masses to their probosces, so that infection can take place by means of insects in the simplest and most natural way from staminate blossoms to pistillate blossoms in these dioecious declinous plants. If the investigator assumes the role of this insect and carries the anther smut to the pistillate blossoms as does the proboscis of the butterfly, he is easily convinced that the smut spores, carried to the inner parts of the pistillate blossom, where they come in contact with stigma secretion and honey, the most favorable substrata for their saprophytic nutrition, germinate most easily here and indeed in the forms described for anther smut in Part V of this work. Nothing stands in the way of the hypothesis that the germ tubes, growing out from single or fused conidia and resembling pollen tubes[2] strikingly in their form, can like these grow through the canal of the style, penetrate into the ovule and, reaching the eggs on the central placenta, can infect them there. Like a flash of

(1) Undoubtedly the whole plant is here attacked by the fungus of the Anther smut, which occurs constantly on all blossoms of the much branched plant.

(2) Compare the illustrations on plate I, Part V, of this work—figs. 25-27.

lightning the thought comes in this hypothesis that in the stigma secretion and in the honey of the blossoms the natural saprophytic substrata are given in which the smut spores germinate, are propagated, and penetrating with their germinating tubes through the canal of the style, reach the embryonic seed. Here again we find an obvious explanation for the facultative parasitism already indicated and for the first exceedingly easy nutrition of smut spores in all possible nutrient solutions. Substrata for the saprophytic nutrition of these fungi are found not only in the soil but also in the blossoms of plants fertilized by insects which are very often attacked by smut fungi. The anther smut of these has as yet been brought forward as one, and indeed as the most pregnant and interesting case for our investigation.

Having discussed these preliminary questions we will turn now to the practical experiments. These experiments were carried out first of all with *Melandryum album*. The pistillate blossoms of these plants were infected with the smut dust from the anthers of staminate blossoms[1]. A suitable brush was substituted for the insect proboscis and the dissemination, that is, the infection of the stigma, was carried through even to the deeper parts of the ovule just as the introduction of the spores is thought to take place by means of the insect proboscis. That infection had taken place was undoubted, but unfortunately the harvesting of seed from the infected blossoms was frustrated. Plants of *Melandryum album* were to be found only outside of Breslau. Therefore inoculation could be made only here and the infected plants could not be constantly observed. They had been cut down when we wanted to harvest the seed. In order to avoid experiences like these, healthy and infected Melandryum plants were grown in the experimental garden. Inoculation could be carried through in the garden and the plants constantly watched. Unfortunately new disturbances became apparent here which could not have been previously suspected. When the capsules had become ripe, it was seen that, except for a small remnant, all of the seed had been eaten up by maggots. This remnant was sown in the following year. Among the plants thus grown was found a number of smutted individuals. Further experience showed that a natural infection can be obtained with certainty, by means of butterflies, when healthy pistillate plants of Melandryum are grown in immediate proximity to smutted stalks. Even microscopic investigation of the stigmas in these plants showed that almost without exception they had been dusted over with smut spores, which observed microscopically, may be seen to germinate on the stigma and to develop further. The seed subsequently harvested from such pistillate blossoms and sterilized, but which nevertheless was strongly injured by insects, furnished as much as 20% of smutted plants in the separate hosts. Their diseased condition could be explained only by the previous infection of the blossoms. We must be satisfied for the present with these details. Investigations like these cannot be completely exhausted in a few years, they need to be continued for many years if they are to furnish results sufficient for all time.

---

(1) In the vicinity of Breslau, we have found only staminate blossoms which had been attacked in the anthers by **Ust. Antherarum.** It has been stated, however, by older authors (TULASNE, GIARD, MAGNIN and others), and held until most recently, that there are also androgynous blossoms. From this it is assumed that the development of these anthers which are always smutted is brought about by the influence of the fungus.

In any case it is of the greatest interest that the two types of inoculation may be found in the smut forms living in blossoms which are determined on the one hand, by the pollenation of plants fertilized by the wind, on the other hand, those fertilized by insects. The fact must be especially emphasized here that the different formations of the smut fungi on saprophytic nutrient substrata as shown in so many cases in Parts V and XII of this work[1], may be harmoniously connected with the infection forms of smut fungi which have now been made known.

(1) Compare the plates in Parts V and XII.

INOCULATION OF WATER PLANTS.

As a concurrent supplement of the above described infection by wind and by insects, water must further be added as a medium and means of infection with smut fungi, which should be taken into consideration in separate cases. The forms of Doassansia inhabit mostly the leaves of water plants; for example, Alisma, Sagittaria etc., and develop in these strongly localized pale spots, in which may be found the threads of the parasite and, especially at the end of development, the large peculiar masses of spores by which the Doassansia forms are characterized. These spore masses consist of fructificative forms only in the inner cells. The outer spore layer is sterile and forms an envelope about the inner spore mass, which thus appears as a morphological entity. These enveloping cells lose their contents in time. These are replaced by air and, when this has taken place, the outer spore-layer becomes a floating apparatus. The spore masses germinate in water like Tilletia[1]. They produce hemibasidia from the separate spores of any mass, on the tips of which, like little heads, is produced a number of conidia. These continue their budding directly and form many filiform bud conidia, both when nourished in nutrient solution and also in water which is not too poor in organic substances. The conidia are formed in great masses. They are separated from the filiform bud colonies into distinct members, which are distributed in the water and can continue their budding even on the upper surfaces. The conidia evidently reach the young leaves which are still submerged, penetrate either under the water or on its surface into these young leaves and, when they are entirely matured and somewhat raised from the water, develop in them the characteristic pale places which betray the presence of Doassansia in the leaves. It is also conceivable that Doassansia conidia reach the mature leaves which are already above the surface of the water. It is, however, not probable that they can penetrate into the already matured and hardened tissues of the leaves. This penetration is limited rather to the meristematic tissues of the plant parts which may be infected, as in all cases of smut fungi. These are the young and immature leaves which in Sagittaria and Alisma are still submerged.

We may say that infection takes place here by means of water, since it is at least very much limited outside the water in the mature tissues of the leaf and perhaps does not occur at all. We must not exceed here a brief mention of the noteworthy infection of host plants of the Doassansiae lest we anticipate the further results of investigation also for the forms of smut fungi living in water-plants; for *Ustilago longissima,* which produces spore masses in the leaves of *Poa aquatica,* and also for *Ustilago grandis,* which grows in brackish water on the axis of *Pragmites communis.* It is probable that the infection of the germinating seedlings takes place successfully in floating media and that the germs of infection distributed from the spore masses through the water reach the young seedlings and attack them. The smut spores are germinated in both cases with hemibasidia, which, according to their formation, were not as yet

(1) See Part XII of this work, plate XII.

definite in form and the conidia of which also grow out to new hemibasidia. These hemibasidia are propagated with the greatest ease in dirty water when organic substances for nutrition are present in it, and they are brought here into natural association with the germinating seedlings. Any kind of infection in these host plants other than the one indicated here is scarcely to be assumed. *Accordingly here in the infection forms of smut fungi there exist three forms of dissemination, by wind, by insects and by water, just as has been proved for the pollen of the blossoms of phanerogamic plants.* It is noteworthy that three forms of infection so important for the etiology of smut diseases have been entirely overlooked and therefore have remained completely unknown.

INFECTION OF THE MAIZE PLANT.

It still remains necessary to subjoin the results of the investigations which were obtained with maize smut and with the smut of Indian millet after the previous publication of Part XI of this work.

So far as the etiology of the smut is concerned, the annual repetitions of infection have given only a confirmation of the earlier results, the fact that all sufficiently young plant parts are susceptible to the germs of infection from without and that the smut itself is strictly localized on the infected places, is confirmed even by the interesting small result that the stigmas of the pistillate blossom spikes, if inoculated when young enough with conidia of maize smut, can subsequently produce smut phenomena. The stigma assumes a garland-like appearance, bends over the swollen pouch-like places and ripens into a small spore mass which forms perfectly ripe smut spores. Such a stigma bundle, deformed by the smut-pockets, forms a highly interesting picture worth noticing, a reproduction of which is not necessary here, since it may easily be imagined. Of course older parts of the stigma are no longer capable of inoculation. One can at most observe the penetration of the germ-threads of the conidia. An effect of infection leading after 2-3 weeks to ripe spore masses can no longer be observed here. The most different varieties of maize were grown for further experimental infections, especially the large form of the horse-tooth maize. In this the infections were less easily successful because it was harder to reach from the outside the sufficiently young tissue, which is tightly inclosed by leaves, meeting together over the vegetative point, and because under the same condition the young pistillate blossom spikes are more tightly closed from the outside by the overlapping of the enveloping leaves, than is the case in the smaller forms of maize. If the opening from above to the pistillate spikes is widened and the fluid for infection with its conidia is introduced, no differences may be seen from the smaller varieties of maize. The same phenomena of smut already described occur here also. Experiments were further carried out, to show that an infection of the young germinating seedlings is one of the greatest rarities. All sufficiently young parts of the matured plant are attacked if they are accessible from the outside for the germs of infection.

We are concerned now only with showing how this infection takes place of itself in nature. As already stated, it does not proceed directly from the smut spores. These smut spores, which are not capable of germination in water, but may do so at any time in nutrient solutions, produce conidial buds on saprophytic substrata, that is, in humus soil, and especially in well manured earth. These conidia very soon pass over to the formation of air conidia[1], which are distributed through the air and are blown on to the maize plants, developing the smut disease.

---

(1) Compare with these the illustrations of the yeast-like conidia and the air conidia, as well as the forms of their growth, on plate IV, Part V.

The earlier experiments have not yet brought out the experimental proof that infection of the host plants is truly brought about by saprophytic centres of infection of maize smut spores which are deposited at a distance from the parts of the maize to be infected. The experiments in this line have been carried out since and have been added to by annual repetition. On lots of young maize plants which had already pushed 3-5cm out from the sheath, which therefore, as young germinating seedlings, had become completely immune, smut spores were sown in such a way, that, mixed with good humus soil, they were carefully sifted between the experimental plants. Then a thin layer of horse manure was put on top and the surface stirred a long time with a suitable rake until the manure was equally mixed with the soil. In this condition, all the lots were left to themselves and further observed. It was shown in all cases where the soil had been sufficiently infected and the content of dampness had been artificially regulated by rain or by sprinkling at short intervals, that even after a few weeks the appearance of smut occurred in the plants and later increased noticeably. All the phenomena of smut reappeared just as they have already been described, in the leaves, in the staminate blossoms, in the axes, in the adventitious roots and subsequently also in the pistillate flower spikes[1]. The places of infection were now arranged as described above, and separated one pace from each other, at varying distances from the lots of maize plants so that the air conidia formed on the soil had to be carried farther to the maize plants by wind from the prevailing direction. It was shown that even here infection takes place by means of air conidia, but that it decreases gradually with the increasing distance of the centre of infection from the experimental maize plants. Restricted by the given special conditions the experiments could not be carried out at a greater distance than 20 metres. It was possible to affirm, however, in each case that a number of maize plants had been reached by the germs of infection carried by the wind and had become smutted. The easy dissemination of the very small air conidia through the air places no limit of infection in nature. Beyond a certain distance, the results become reduced and only isolated instances of disease occur. Certainly the universal distribution of maize smut is chiefly promoted by air conidia, if not entirely by them. If this be true, the overcoming of this smut can be attained only by burning the smutted plants before they have allowed their smut spores to reach the soil; for its infection proceeds always from the soil and the spores dropped from smutted plants on to it are later the natural centres of infection for the increase of the disease.

Further, the experiment was not overlooked of collecting and sowing the still healthy grains of maize spikes, which had been attacked by this smut only in the uppermost parts. It was shown, as might have been presupposed, that this grain, sterilized before sowing, brought forth perfectly healthy plants and that in the interior of the grains no vegetative fungus was present. Of course the smut can be carried over in the seed of maize taken from smutted fields, by the spores which cling to the outer surface. These spores get into the soil and, if it is impossible for them to attack the young maize plants they may still become centres of infection which can bring about a renewed infection from the soil by saprophytic nutrition and by

(1) See plates III-V, Part XI.

the development of air conidia. On this account it is advisable to sterilize impure seed of maize in order to destroy thereby all smut spores clinging to its surface.

If we take into consideration the fact that air conidia of the maize smut lead to infection of the mature host plant and that they alone may bring it about, that these air conidia, driven by the wind into the openings and rifts of the host plants, attack the young tissues to be found there and make them smutted, it then becomes self-evident that maize plants which, by enveloping leaves, shut off all young tissues, susceptible to attack externally, must be at the same time the most resistant to this smut. These are the large varieties, to which belong especially the horse-tooth maize. It must be just as self-evident that the usually smaller varieties of maize, in which the leaves open over the vegetative point like a paper sack and in which the pistillate flower spikes are less protected by their husk leaves, show a marked susceptibility to the smut. The experiments described above and reported in Part XI of this work[1], were made accidentally with a smaller variety of maize which is especially suitable for experimental infection. Only later comparative experiments with other maize varieties showed clearly how these are protected by the above named accessory conditions from the blowing in of the germs of infection and how it must naturally appear that in this variety the smut is formed only rarely.

(1) Plates III-V.

INFECTION OF INDIAN MILLET.

In passing over now to the smut of Indian millet, to *Ustilago sorghi (cruenta)*, we have a different form of disease in host plants, which forms spore masses exclusively and only in the inflorescences. Infection most undoubtedly takes place here generally in the germinating seedlings of fresh seed, even when the spore masses appear first in the flower panicles of the mature plants. In earlier experiments, when infections were carried out in sufficiently young germinating seedlings, as high as 70% of smutted plants was obtained. In later experiments these results were repeated in approximately the same way[1]. However, it was assumed earlier that infected plants could not develop spore masses if they outgrew the smut germs by too rapid development so that these could not reach the vegetative tips. However, in all cases it could be proved that infection had taken place in those plants by the fact that fungus mycelia were shown in the nodes of the grass and in the parenchyma cells and that it had been retarded and had not reached the vegetative point nor passed over to the formation of spore masses[2].

In order to prove that infection had actually taken place, these plants were cut back to two thirds of the height of the axis, as soon as it was seen that the panicle of the tip was healthy. By this means the formation of axillary sprouts was caused and this formation takes place, as described earlier in Part XI, at those points in which mycelia exist enclosed in the parenchyma cells of the nodes. By this new formation of tissue for the axillary sprouts, parenchyma cells were affected which harbored the mycelia. These can here penetrate through the young tissue and reach the vegetative points. It is now seen that in accord with the experiments carried out previously with other plants, these axillary sprouts became smutted. Therefore smut can be brought to development in apparently healthy plants as if by a stroke of magic, if the apical healthy inflorescence is removed early enough, thereby causing the possibility of an axillary sprout formation. In our climate this occurs very rarely in sorghum plants when not artificially introduced. It can, however, take place, and with the result that the smut infection already existing will be subsequently proved by the smutted axillary sprouts. However, these earlier experiments are capable of being multiplied from still another point of view. If it is true that smut germs, which have already pressed their way in, are frustrated by too rapid growth of the host plants and that thereby the decrease in the percentage of smutted plants is brought about, the question arises whether this too rapid development of the host plant cannot be restricted. This is most easily possible if seed older than that of the previous year is used, the grains of which have weakened more or less in germinating energy with increasing age. The young germinating embryos, whose development is retarded by delayed germination, are most excellent material for further experimental infection of the young germinating seedlings. This inoculation was carried on by means of an atomizer by the spraying with spores which had stood a day in nutrient solution and which thus were brought to direct germination. It was

(1) Compare the text of Part XI, pages 43-51.

(2) Compare fig. 7, plate I, Part XI.

proved in the autumn that a total infection of the host plants had taken place here[1]. Unfortunately such a totally infected field of Indian millet cannot be photographed, because the smutted plants of these experiments are not conspicuous enough to appear as smutted ones in a photograph. Imagination suffices to form a clear picture of this most striking phenomenon.

Further, the question is still unanswered whether in the smut of Indian millet infection of the blossoms cannot also occur. Inflorescences are found which are totally infected. These are usually the first to appear. Then follow other inflorescences which show only partial infection and in which, between the smutted blossoms, may be found others blooming normally and provided with stamens and ovules. The sorghum smut is not as dusty as the loose smut, but it can be blown with ease into the partially attacked inflorescences, or those perfectly healthy. Unfortunately these experiments had no decisive results, because Indian millet in our climate ripens in separate heads only in especially favorable vegetative years and forms ripe grain only rarely. Therefore it can not be decided with certainty how far infection of the blossoms takes place here. In only one case could healthy grains be gathered from partially smutted panicles in which a spraying with the smut spores had been made use of. From these grains, however, healthy plants were grown.

---

(1) The total infection obtained here gave ground for carrying out the experiments in the same way for wheat and barley with seed of different ages. As may be seen from the survey of infection experiments given in detail pages 26-30; the experiments, however, were unsuccessful.

INFECTION OF PANICUM (RISPENHIRSE) AND ITALIAN MILLET.

Besides the two plants experimented upon, maize and Indian millet, with the corresponding forms of smut, during the last ten years two other experimental objects were grown supplementally which are especially well suited for the purpose of infection. They are first Panicum with *Ustilago Panici miliacei (U. destruens)*, and then Italian millet with *Ustilago Crameri (U. Setariae)*.

In Panicum the occurrence of smut is especially characteristic in its external appearance. In the plants attacked, the otherwise loose, long, panicle-like inflorescence is shortened as much as possible and all the single attacked blossoms united in a smut gall, which is enclosed by sheath leaves. These leaves have undergone a complete fungus pseudomorphosis from mycelial threads which have remained sterile and form a dazzling white envelope about the thick clump of spore masses. The smut galls are sunken within the unchanged green leaves of the upper axis and are only a very little exposed to the open air. The plants attacked, in contrast to a healthy one with its long, outstretched blossom-panicle, make an entirely different impression, so that they may be recognized in the field even from a great distance. The black spore masses in the interior of the gall are not disseminated. They are, however, easily germinated in nutrient solution or in water and produce four-celled hemibasidia, in which a formation of conidia occurs, sometimes to a lesser, sometimes to a greater amount[1]. The conidia very rapidly grow out to germ tubes and form in dilute nutrient solutions small mycelia, on the threads of which may be observed the formation of air conidia. Further particulars are to be found discussed in detail in Part V of this work.

The ripened smut spores, sieved and well-preserved throughout the winter, are purified on a centrifugal sieve and result, after twenty-four hours' retention in dilute nutrient solution and also in water, in a directly aggressive material for infection. One can follow the rapidity with which the spores germinate and form hemibasidia with conidia, which in turn grow out to germ tubes. Inoculation was undertaken on previously chosen grains of Panicum, in which germination had just started, by means of the atomizer and the cultures, protected from the light, were set back in a moderately warm place. After the lapse of a few weeks, the cultures were opened, at first still protected, in order that they might be planted. In infected plants in a series of experiments extending over several years, an average of 60-70% of smutted plants was harvested. The result in healthy plants could be explained easily by the fact that the comparatively small germinating seedlings of Panicum offer only a limited surface for the spraying on of the germs of infection. However, the discoveries obtained previously with Indian millet were established here also, in obtaining a percentage of smutted plants by delayed development of the host plants, that is, of the young germinating seedlings. Seed from former years was used for the experiments and it was determined with certainty that a slower germination took place here, in which infection was carried out by means of the atomizer in the way described.

(1) See illustrations on plate VII, Part V.

Varieties of millet with black grains and with white ones were used. The result, which continued to be the same in succeeding years, was the most favorable conceivable, namely, a total infection of all the plants under experimentation. The variety of millet with white grains is best suited to give striking and beautiful illustrations of smut phenomena. The plants obtained a height of more than 4 feet and the galls of smutted ones became a large as a walnut.

An experimental object more favorable for the inoculation of young seed than that existing here in millet is scarcely conceivable. It is still undecided, however, whether infection takes place only on the young germinating seedling or whether it can also take place successfully in the blossoms. The proof of air conidia in this form favors the infection of the blossoms of the host plant. The single blossoms of these host plants are, however, so small that the probability of blossom infection is thereby greatly reduced. It should be added to this that the smut galls in experimental fields are not disseminated and therefore could not succeed in reaching the soil directly, so that any direct formation of air conidia is thereby practically prevented. Infection must then have taken place from the conidia of a saprophytic nutrition. They can be formed on the upper surface of the soil from spores which had been previously disseminated. The probability that this may happen is not great. Still greater, however, is the other probability that in sowing millet in the open air the young germinating seedlings are reached by air conidia. Practical experiments on the inoculation of the blossoms resulted negatively. Infection of the blossoms is, however, in no way excluded by this, but is reduced to a minimum.

Experiments with *Setaria Italica* with its smut forms had about the same results as those described above for Panicum. A black shimmer may be seen in this thick, club-like inflorescence, already infected, when the ripe ovules rupture, freeing the smut. The black smut spores are not disseminated. They usually remain so enclosed in the thick panicle of Italian millet that close examination is necessary in order to recognize plants attacked by smut. It often occurs here that only a part of the blossoms of a panicle are attacked and that normal blossoms free from smut may be found between the diseased ones. The smut spores in any case germinate into four-celled hemibasidia, in which often no conidia at all occur, which, if they do appear, however, grow out very quickly into germinating tubes. Air conidia have not been observed in cultures of these spores. Smut spores, obtained pure in the autumn, germinated in the spring, easily and surely, especially in dilute nutrient solutions. They were used further only after purification with a centrifugal sieve and after one day's retention in dilute nutrient solution. They were sprayed on the seedlings of Setaria which had been thus prepared and were just germinating. The cultures were treated as above and the infected seedlings, when they had reached a sufficient size, were planted out of doors. These seedlings are exceedingly small, so that one might suppose inoculation by spraying with spores would have no result in the experimental plants. Experience, however, proves the opposite; as high as 70% of smutted plants were obtained and it was possible to achieve here, as in Indian millet, and Panicum, a total infection, by using for this infection seed which is somewhat older and therefore sprouts more slowly.

With the absence of easily disseminated spores and also lack of air conidia, blossom infection is here from the very beginning improbable. This could be promoted only by the special circumstance, that in partial infection of a flower spike, healthy blossoms may occur between the smutted ones and therefore be directly adjacent to these. The possibility thus given for blossom infection could be easily tested by harvesting the ripe seed from partially smutted spikes and using it for sowing the following spring. The results of these experiments gave no smutted plants. Accordingly, the probability of infection in the blossoms in any case is very slight, if it exists at all.

In the experimental plants last treated, that is, Indian millet, Panicum and Italian millet, we have varieties and host plants for smut fungi in which *the infection of the germinating seedlings may be considered as the prevailing type of infection in smutted plants, if not the only one,* while the infection of the blossoms, if it takes place here at all, seems to be limited to a small amount.

Spraying with the germs of infection by means of an atomizer was especially suitable for carrying out the infection of the young germinating seedlings, if the precaution was taken of purifying the spores with a centrifugal sieve and of preparing them for direct germination by one day's retention in dilute nutrient solution.

Further plants for experiment have not been taken up as yet in our investigations and experimental infection. The exceedingly important smut forms of the stinking smut of wheat and the covered smut of barley have not been overlooked, but at first only several experiments could be arranged, because it was impossible to undertake and observe too many experiments at one time and because it was better to await the results of those already undertaken in order to use them explanatorily when making others.

Blossom infection with stinking smut of wheat and the covered smut of barley were begun last summer, however, and will furnish results only in succeeding vegetation periods.

FINAL CONSIDERATION.

From the preceding experiments as a whole, it is obvious that the previous assumption of a successful infection of smut fungi limited only to germinating seedlings is not universally satisfactory. Besides infection of the young seedlings still other forms of infection exist which had been overlooked.

We can report in general that only the *youngest embryonic tissues* of the host plants are the ones attacked by the germs of infection. The germs of the smut fungi have no power of attacking older parts of plants the tissues of which have become hardened.

Experiments on the infection of maize smut proved clearly that the large host plants, in the case of their development and formation, expose in different places the youngest embryonic tissue to the attack of infection germs of maize smut. These points of attack extend even to the embryonic pistillate inflorescences which usually appear only after the complete maturing of the plants. In maize the young leaves of the vegetative tip, the staminate inflorescences and the young axes may be reached by germs of infection, blown upon them. In the same way also infection of the pistillate inflorescences and adventitious roots takes place in the host plants. That infection has taken place is easily and surely determined here and the appearance of smut occurs after the lapse of perhaps three weeks. The smut remains localized upon the separate places in which inoculation was successful and occurs independently on all the above-named places which are susceptible to smut germs and capable of infection by them.

In the other smut forms occurring in our grain the matter is essentially different. The phenomena of disease do not develop in the parts in which infection has taken place. The effect of the infection is shown only after a long period of incubation, after many months, with the unfolding of the inflorescences. In the inflorescences alone are provided the only places for the formation of spore masses and these inflorescences lie at the opposite end of the host plant from the one attacked in the first stages of the germination by the germs of the fungi. The host plants during their whole life are surrounded externally by mature and hardened tissues, into which the germs are not able to penetrate. The host plants only once and indeed only at the beginning of their development offer external young tissue to the germs of infection. These are the first germinating stages of the young seed in which infection must take place in the soil, if smutted plants are to be produced subsequently. These facts correspond throughout to the previous and older theory that infection of smut fungi takes place in the young seed. This undoubtedly happens, but in thus judging it, the fact was overlooked that host plants, at the time of flowering, offer again in their ovules and stigmas very young and assailable tissue for the germs of infection and that infection can take place in the embryonic parts of the pistillate inflorescences as well as in the young germinating seedlings in the soil.

Thus our investigations have produced certain proof, that this infection actually takes place in the blossoms and that, carried by wind or insects, the smut dust is brought from smutted individuals to healthy plants.

This very obvious example of infection in the blossoms had been hidden and kept from a full understanding by the fact that the smut in the infected blossoms did not appear in the same year with the ripening of the grain; that rather the germs of infection which have penetrated into the seed lie latent there and develop in the motured and blossoming plants only in the following year, with the germination of the seed. Blossom infection, however, which could be proved with certainty in the pistillate spikes of maize occurred here therefore in a varied form, in that the period of incubation up to the breaking out of the disease, that is, until the spore masses mature, is considerably longer. It is not sponned by three weeks, but is completed only in the second year, following the finished inoculation. In this noteworthy foct lies the peculiarity of the now ascertained blossom infection of our varieties of grain.

Accordingly, in the occurrence of smut diseases in our grains, we must reckon with two places of infection quite independent of each other; first, the young germinating seedlings, second, the blossoms. We must consider that in separate cases both forms of infection may be effective at the same time, but first one ond then the other will be predominantly active. In judging of the natural spread of smut fungi and smut diseases, these recently explained facts are of decided value.

However, in the details here given only the development of the parasites *within* their host plants has been considered, the different forms in which infection takes place and also how, from the germs of infection already present, the further development of the smut fungi and of the phenomena of diseases is carried up to the formation of spore masses.

Now, however, by means of the earlier investigations and cultures reported in Parts V, XI and XII of this work, it has been proved that smut fungi can live not only *in the host plants,* but that they occur also *outside of the host plants* on saprophytic substrata and mature there *in different and new forms,* which have not been observed within the host plants. However well smut fungi, as parasites in the host plants, may show the most complete adjustment to their hosts, which adjustment can be observed only in nature, they are rather not specific parasites, but only facultative ones. They may live and flourish outside the host plants in all substrata to be found in nature. A rapid and active development of the smut fungi takes place in these nutrient substrata and especially an exceedingly abundant increase of the germs. Smut fungi live in nature outside the host plants just exactly as do other saprophytic fungi and their propagation takes place especially where nutrient substrata are present in humus and well manured soils. A saprophytic development results here and also a propagation of the germs. From these places, as in maize smut where air conidia are formed, the germs of infection can be distributed on to the susceptible parts of neighboring host plants. In other cases where air conidia are absent, the germs of the smut fungi, developed and increased in the soil, will attack the young germinating seedlings and produce the phenomena of smut.

Further, nutrient substrata, independent of soil, for the development of smut fungi, may be found in the secretion of the stigmae and in the honey of plants fertilized by insects. In all such cases saprophytic nutrition of the smut germs introduced in these places may be proved with certainty.

According to the earlier hypotheses, infection was dependent on the direct products of germination of the smut spores; therefore, so to speak, on these alone. It was assumed from the weakly germinating smut spores that they inoculated the germinating seedlings and that from these inoculations smutted plants were produced in our grain fields. This theory, however short and convenient for the explanation of smut infection, has been supplemented by the proof of an extensive distribution and propagation of the smut germs in saprophytic substrata outside of the host plants. Only by determining this has the way, in which the germs of infection are distributed, become clearly and certainly understood, as well as the natural infection and distribution of smut germs as observed universally in nature. The biological section with the development of smut fungi on saprophytic substrata outside the host plants, forms, according to our present understanding, the complement of the section with development taking place in the host plants. Both parts are now united in a harmonious whole and nothing can characterize this harmonious union further and more sharply than the fact that, for instance, in the forms of the genus Ustilago, at the time of parasitic life in the host plants, only chlamydospore fruit forms, the typical smut spores, mature and that during the period of saprophytic nutrition only conidia fruit forms appear. For this strict alternation in the maturing of the fruit forms which takes place here, not, as in the case of the Uredineae, on two different hosts, but after saprophytic and parasitic nutrition, we can for the present find no other explanation than the influence exercised on the development of our plants, at one time by the living substrata, the next time by the dead substrata. How would it be possible, according to the earlier conceptions and the earlier knowledge which had not led even so far as to the germination of the smut spores, to explain the phenomena in maize smut and to understand them correctly, if the portion of the development of smut fungi enacted saprophytically did not furnish the natural explanation for all details? It is scarcely possible to find anywhere in the whole domain of infectious diseases a more complete or finer picture of this most striking phenomenon as it exists most clearly here in the etiology of the maize smut. And not less clear has become the understanding of the smut forms living in our grains which propagate their germs of infection in the soil by saprophytic nutrition and especially in manured soil, in such a way that infection of the germinating seedling may thereby be understood and the significance of manure for the occurrence of smut diseases in grain as agriculturists have always emphasized is shown in the proper light.

Certainly, however, the phenomena of blossom infection are not less convincing and clear. In them the smut germs find their nutrient substrata in the secretion of the stigma and the exudation of honey, which are as favorable as possible for germination, development and propagation of the germs of infection.

It took a long time, more than the lapse of twenty years, to make possible the obtaining of the explanations here given concerning the biology of the smut fungi, their infection, the phenomena of the disease and the natural distribution of smut fungi on saprophytic substrata. It was not easy to find in the separate cases the right road which would lead to this goal.

It should be noticed here, however, that the universal end of the new investigations, however successful they have proved to be in the cases already carried on, has not in any way been reached and that still many separate experiments must be carried out in order to obtain the

results made possible by this newly acquired understanding of the matter. The investigations are therefore as difficult as they are wearisome and experimental infection encounters impediments in the undertaking and in its carrying out which are scarcely imaginable in advance.

The peculiarity of these experiments on infectious diseases in plants is that they can be carried out in part only with the resources of an institution; and in part, moreover, only with those of an experimental field in which the cultures of inoculated plants must be brought to a finish. The undisturbed harmonious co-operation of these two factors, the arranging of the experimental field, the preparation and the work in the institution, is possible only if the experimental field and the work rooms of the institute are as closely and conveniently connected as possible. Only thus is it possible to observe the cultures for any length of time and to keep away the many kinds of external disturbances to which they are exposed in the course of the period of growth.

The chief impediment to the rapid progress of investigations and experiments in this direction lies, however, in the circumstance that, during the whole length of time of one growing period, experiments can be made only once, the results of which are given only at the end of the summer. If these experiments have been disturbed by secondary and other injuries, or if they give only negative results, a whole year is lost before the experiments can be renewed and supplemented. Thus several periods of growth are often needed for the deciding of simple questions and the end of the experiments can be reached only years later, by the dispatching of questions, possible only from time to time. In this way it becomes obvious that the investigations reported here have not been absolute, even in the most favorable cases, but are only relatively conclusive. In many places, the points in question have been left open where the results already obtained from the cultures have not been sufficiently decisive. Years must still elapse before one can speak of universally conclusive results.

If one considers this state of affairs and the unusual circumstances which come into consideration in the experiments and their carrying out, one will involuntarily be led to think that an arrangement suitable for the investigation of smut diseases and similar infectious diseases would be most opportune. If only the damages which are annually caused by the smut of grain be compared with the expenses of an institution of the kind indicated, this small material sacrifice would certainly not be proportionate to the prospective advantages of an explanation of the natural spread of smut diseases and the successful struggle against them. But here the external resources of an institution and of the experimental field are not primarily concerned. Even if these are granted no favorable results can be obtained when there does not lie at the disposal of the management of such an institution a power broadly educated in the understanding of the matter and also mycologically.

OF THE ASSIMILATION OF NITROGEN[1].

In the inoculation of blossoms with the loose smut of barley and of wheat it has been proved possible to harvest grain which after sowing gave total infection of all plants under experimentation. In the same way in the smut of Indian millet, Panicum and Italian millet, a total infection of the plants under experimentation was obtained, if the germinating seedlings were inoculated sufficiently carefully by means of an atomizer. We have accordingly in the host plants of the smut fungi here named material which will lead with perfect certainty to the formation of smutted plants.

With this material it has now been possible to decide definitely a physiological question of especial interest; namely, the question as to a possible assimilation of free nitrogen by the fungus mycelia which live parasitically in their host plants. This question became of importance through the excellent investigations of *Hellriegel,* which prove definitely that lupines and other Leguminoseae can live in soil without combined nitrogen, that is, chemical compounds of nitrogen, and are able to assimilate the free nitrogen of the atmosphere, if they are inhabited by parasitic fungi. *Hellriegel* succeeded in bringing the above-named Leguminoseae to full development in pure vitreous sand, which had been provided with mineral salts in solution, but remained free from combined nitrogen, if definite forms of bacteria rhizobia could attack the roots of these host plants and produce tuber-like swellings there.

The fortunate results of *Hellriegel's* cultures have brought forward the question whether other fungi living parasitically in their host plants can cause a similar assimilation of free nitrogen. A series of phenomena, for instance, mycorhiza, which occur universally distributed on the roots of different plants, favors its explanation in this way. Experiments were made with tree-like plants several years old, in which it was thought possible to prove that the mycorhiza living on the roots can cause assimilation of free nitrogen. Experimental material in perennial, slowly growing plants, said to assimilate uncombined nitrogen, does not promote, however, the decision of this question as to the assimilation of free nitrogen. In time, unavoidable sources of error creep into experiments with perennial plants, which offer no security for a scientifically certain result. Experiments of this kind can be carried through successfully only with quickly growing and large annual plants through which the proper parasitic fungi grow, from germination to the end of development, and which attain the most luxuriant development possible. In this kind of experimental object, it must be assumed that parasites of these plants living saprophytically do not cause the least damage and that some connection exists between the parasites and the host plants, as was found in the Leguminoseae and their rhizobia. Experimental objects of the necessary and desired kind are furnished now in an absolutely ideal form in our large annual grain species which smut fungi attack and in which they live. In one vegetation period a plant attains its complete size and maturity. The parasite penetrates into

---

(1) A preliminary report on this subject has been published in a lecture before the Schles. Gesellschaft für vaterländische Cultur, on the 15th of November, 1900.

the plant in the first embryonic points, continuing its growth with the maturing of these, until at the end of development they pass over in the blossoms to the formation of spore masses. The adjustment of the parasite to the host plant is the most complete possible. Nothing at all of any appearance of disease is to be seen in the course of the whole development of the host plant up to its complete maturity. In fact the phenomena are constantly repeated, that the host plants attacked by fungi develop more quickly and luxuriantly than healthy ones and that spore masses have appeared in them when the inflorescences are just beginning to show in healthy plants. One is tempted involuntarily to believe that parasites living in infested host plants can exercise an influence favorable for a quick and complete development. These external circumstances make somewhat desirable the choice of these experimental plants as objects for the decision of the question whether fungus threads living parasitically in their host plants, here especially smut fungi, are able in connection with these to assimilate free nitrogen and thereby be in a position to cause a more luxuriant nutrition of the host plant.

Up to this point only one impediment had been found in using host plants attacked by fungi for this kind of experimentation and this lay in the circumstance that in the experiments it was never certain whether the plant under experiment had been attacked by smut and whether one was actualy working with smutted experimental objects which alone could bring about a decision of the question. This impediment has now been overcome by the continually improved methods of inoculation of host plants with smut fungi. It became possible in the different millet forms,—Indian millet, Panicum and Italian millet,—to produce with certainty infected germinating seedlings for the experiments and just as surely to use seed obtained from blossom infection which from experience was seen to produce only smutted plants.

The natural method of arranging the details of the experimentation works out of itself,—as *Hellriegel* had planned it. According to him, sterilized absolutely pure vitreous sand was saturated with mineral nutrient solutions, but without any nitrogen compounds, and then put in glass jars, which, sunken in soil, were provided with openings and a covering of gravel for purposes of ventilation. In this substratum the freshly infected germinating seedlings of the three millet forms,—Indian millet, Panicum and Italian millet,—were planted in separate pots, from 3-5 specimens being put in each. The single plants were weighed on the decimal scales at the beginning of the experiments and daily loss through evaporation was replaced with distilled water free from nitrogen. For comparison, pots were arranged in the same way as those described above, only with a corresponding addition of a nitrogen compound in the form of calcium nitrate. In these parallel experiments the same number of experimental plants, that is, germinating seedlings, were planted in each pot. The experimental plants were then placed under protection in the propagating house and in good weather carried into the open air on a truck in order to expose them to the direct sun. Thus, with this method of setting up the experiments, sources of error could not creep in. The transplanted seedlings in both series of experiments grew without difficulty in the vitreous sand and in the first eight days showed scarcely noticeable differences. Then after the exhaustion of foodstuffs in the germinating seed, the lack of nitrogen on the one hand and the action of the nitrogen compound on the other, first made themselves felt and led to an even more striking phenomenon. In the next four weeks the pots without nitrogen

compounds showed scarcely any advance in the young plants, while the plants in the pots with nitrogen compounds matured daily more luxuriantly. After six weeks the contrast was as great as possible. When no further advance in the development of the dwarfed plants without nitrogen compounds could be observed, a correspondingly slight amount of nitrogen compounds was introduced in the sand in the solution. Even in the next few days the effect was apparent. The little plants developed further and, after the lapse of about three months, so far as their size, even if dwarfed, permitted, the formation of blossoms could be recognized. When these had matured it was shown that all experimental plants had become smutted, as might have been presupposed. Also in the comparative series of experiments abundantly provided with nitrogen and in which nitrogen compounds had also been added subsequently in order to cause the greatest possible development, the plants did not remain in size much behind those in the open field and showed in the inflorescence development of all individuals the most luxuriant formation of smut. By means of photographic exposures (made by *R. Scholz*) the two parallel experiments of the Sugar millet (Zuckerhirse) have been permanent and are reproduced in fig. 1, plate 2, of this volume.

The result of the comparative experiments shows most conclusively that fungus threads living parasitically, in this especial case, of smut fungi, are not able to bring about the provision of their host plants with nitrogen from the air. Without nitrogen compounds, they soon stop growth and resume the phenomenon only when further nitrogen compounds have been added. From a comparison of the dwarfed plants without nitrogen compounds with the luxuriant normal forms obtained by means of them, it is seen conclusively that filiform fungi, living parasitically and concerned here, are *not* in a position to bring about an assimilation of free nitrogen even when the most favorable objects are grown for the experiments. If assimilation of nitrogen cannot be proven here, there is no great possibility that it can be the case in other fungi living parasitically. The somewhat quicker and more luxuriant development of host plants attacked by fungi, mentioned above, must have other subsidiary causes which, however, in any case may not be traced back to nitrogen assimilation.

Experiments with these millet varieties were repeated in the course of three years and always with similar results. Wheat and barley were used only later for the same experiments.

Instead of young inoculated host plants, the experimental pots were sown with grain which had been kept over from previous harvests, in which there had been a total infection of the harvested grains. The experiments were set up and carried through in the same way as those described above. During the period of experiment no disturbances whatever occurred and the results were exactly the same as described for millet. The grain germinated into healthy seedlings, which, lacking nitrogen compounds, stopped growing after all the reserve stuffs had been used up. When in the course of three or four weeks this cessation of growth took place, the plants were so benefited by a single addition of nitrogen compounds that they formed small inflorescences, which in every case were entirely smutted. The comparative experiments with nitrogen compounds again gave a normal development of the plants and also the formation of completely matured smutted heads. By photographic exposures the actual condition was made

permanent and visible here as in millet. The series of experiments with infected barley and with wheat substantiated in short the earlier results with millet. In this can be recognized no proof that any assimilation of free nitrogen takes place, caused by parasites in the host plants, and we can say directly that the host plant infected with smut is here exactly as dependent on nitrogen compounds as are other healthy plants. The negative result of these experiments makes the facts ascertained by *Hellriegel,* for Leguminoseae, appear so much the more prominently. The subject is finally limited to the fact that according to our present knowledge only the rhizobia possess the capacity of bringing about assimilation of free nitrogen to any great amount when living parasitically on the roots of Leguminoseae.

EXPLANATIONS OF THE ILLUSTRATIONS.

Plate I.

Fig. 1. A small totally smutted experimental field of summer barley, grown from sterilized seed, which, as blossoms, had been inoculated with fresh smut spores. Many heads have become only partially smutted.

Fig. 2. A similar experimental field of summer wheat. The few healthy heads belong, however, to smutted stalks.

In the text this fig. 2, as a result of an inadvertent reversal of the pictures, has been designated as fig. 1.

Plate II.

Fig. 1. Smutted experimental plants of the Sugar millet, which were inoculated as young germinating seedlings. The pot at the left contained all kinds of nutrient salts, including calcium nitrate, the one at the right, no combined nitrogen. In the latter the experimental plants, after the addition of calcium nitrate solution, developed and formed blossoms, all of which were smutted.

Fig. 2. Smutted experimental plants from sterilized two-year old wheat grain which two years previously were inoculated with smut spores at the time of flowering. The right hand pot shows the development of smutted individuals in pure vitreous sand containing all kinds of nutrient salts, including calcium nitrate. In the left hand pot development took place at first without the addition of combined nitrogen. Later, however, a slight amount of calcium nitrate was added in order to further the development up to the formation of blossoms. In this part also all individuals were smutted.

PLATE I. FIG. 1.

PLATE I. FIG. 2.

Completely Smutted Experimental Fields of Barley and Wheat grown from Sterilized Seed inoculated when in Blossom.

PLATE II. FIG. 1.

PLATE II. FIG. 2.

Completely Smutted Experimental Pots of Sugar Millet and Wheat with and without the Addition of Combined Nitrogen.